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CONTENTS

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Vernon Boyson, *Managing Editor*

THE AMERICAN NATURALIST is a bimonthly journal devoted to furthering the objectives of The American Society of Naturalists, which are the "discussion, advancement and diffusion of knowledge concerning the broader biological problems, including organic evolution, thus serving to correlate the various biological sciences into a common philosophy of biology." It will publish those general addresses, essays and papers presented at the symposia of biological societies which contribute to the above purpose; research papers and reviews in which theoretical interpretation and synthesis are predominant; and brief discussion, criticism and comment on material published in this journal and elsewhere. "Letters to the Editors" containing new information or comment will be dated when received and published in the next issue. They should not exceed two printed pages.

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Annex 15

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IN MEMORIAM

The AMERICAN NATURALIST has for many years benefited from the enduring interest and high professional competence of the Cattell family. In 1938 Jaques Cattell became editor, and in 1951 he established a relationship with the American Society of Naturalists providing for publication of the Journal in the interests of the Society. His broad experience as an editor, publisher, and counselor of science provided him with unique qualifications to continue the high standards set by his predecessors.

Jaques Cattell was interested in science, and in the men who contribute to it. He wished the NATURALIST to provide in its pages a place for speculative essays, correlation and synthesis. With his guidance and knowledge, the AMERICAN NATURALIST maintained a high standard among scientific publications. The Journal was truly a source of pride to him, rather than a business venture.

Mrs. Jaques Cattell will continue to publish the AMERICAN NATURALIST. Traditions accumulated by the NATURALIST in nearly one hundred years of publication, and enriched by Jaques Cattell's association with the Journal as its publisher, will be followed as closely as possible according to his wishes.

VERNON BRYSON
Managing Editor

THE
AMERICAN NATURALIST

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DIVERGENT RESPONSES TO SELECTION BY TWO POPULATIONS OF *DROSOPHILA MELANOGASTER**

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Since the domestication of *Drosophila melanogaster* about half a century ago, its many keepers have known that its eggs hatch the day after they are laid. With somewhat greater precision, Poulson (1950) puts the hatching time at from 22 to 24 hours at temperatures between 23° and 25°C. More detailed data, if ever collected, do not seem to have been published and nothing is available on whether different populations differ in hatching time. As part of a study of differences between populations in developmental rates, a very considerable body of data has been collected on the hatching time of several populations. These data provide information of a sort previously unavailable. Among them are evidences of interesting differences between populations and of even more interesting changes in these differences.

METHODS AND MATERIALS

To measure hatching time one must have eggs laid within a short interval and one must record the number that hatch within each of a number of short intervals around the mean. The most practical number of eggs for a single sample is 50. It becomes very difficult to record hatching accurately for larger samples. To be reasonably sure of obtaining samples of 50 eggs laid within a 20 minute period—the interval found most feasible—one needs between 100 and 150 female flies. For this purpose about 250 flies of both sexes were shaken without etherization into an empty half-pint milk bottle and the eggs to be used for the measurement of hatching time were collected by placing a thin slice of agar on the under side of a bottle cap, inserting it in the bottle containing the 250 flies, inverting the bottle and then replacing the cap and wafer every 20 minutes. Eggs to be observed at hatching time were set out in five rows of ten on a fresh slice of agar and kept in a Petri dish. Observation began between 17 and 18 hours after mean laying time, the mid point of the 20 minute interval during which the eggs were collected, and was continued at ten minute intervals to 24 hours.

*This work was supported by a grant from the National Science Foundation, G-8715.

There are reasons for believing that the hatching distribution of *D. melanogaster* deviates from the normal and that some populations deviate in different and characteristic ways, but to demonstrate this requires very large samples obtained by consolidating the counts on many groups of 50 eggs each. For a 50 egg sample the distribution appears close to normal, especially for one standard deviation on either side of the mean. Hence the most rapid and convenient way of analyzing these data is to convert them to cumulative percentages and plot these on normal-ruled graph paper. There is usually little difficulty in fitting a line by eye and from this one can read the mean and standard deviation.

In making these measurements one encounters a very troublesome fact. A female fly sometimes retains an egg in the uterus after fertilization, all the way to hatching time on occasion, but she may deposit it at any time between. It is obvious that if many eggs retained for various periods are included in a sample of 50, the mean hatching time will bear little relation to the time of development. A held-back egg which hatches four hours after mean laying time can easily be spotted and deducted from the sample. One which hatches 60 minutes before the mean is not certainly identifiable as such. But one is, of course, not justified in truncating the beginning of the distribution to make a Procrustean normal. A compromise has been effected by rejecting all early hatchers which are separated from the remainder of the distribution by 50 minutes or more. The number of held-back eggs can be greatly reduced and the problem fairly well obviated by setting up the sample to be observed from eggs obtained after the parent flies have been laying well and steadily for some time—usually at least three consecutive 20 minute periods.

Among the populations on which measurements of mean hatching time have been made are two large, randomly breeding populations maintained in cages since 1953. One of these, Syosset (Sy), originated from about two dozen flies collected in a grocery in Syosset, New York, in July, 1952. For a year it was maintained in bottles in mass culture. The other (OR) stems from the well known Oregon R stock which has been cultured in numerous laboratories for many years. The present cage population was set up in 1953 from a mass culture which had originated from the stock maintained at the Department of Genetics of the Carnegie Institution of Washington, Cold Spring Harbor, New York. In addition to the two large populations, three inbred strains have also been measured—SySP, I_1 and I_2. The first of these stems from Sy and has been carried by brother-sister mating for over 200 generations. The other two stem from OR and have been similarly inbred, separately, for well over 100 generations.

Flies whose eggs are used for measuring hatching time are raised in half-pint culture bottles so seeded as to produce about 300 flies per bottle. In the case of Sy and OR these bottles are seeded with eggs laid on a fresh food cup in the cage. For the inbred strains, since the usual offspring of a single pair is only about two dozen flies, and since a substantial percentage of their eggs do not hatch, the offspring of several pairs are placed in a

culture bottle and then transferred every two days to a fresh bottle. The flies which grow up in these bottles are used for egg collection. Whether the bottles are seeded with eggs from a cage (Sy and OR) or with eggs laid by flies in the bottle (SySP, I_1 and I_2), about a teaspoon full of grated baker's yeast is added to each bottle when the larvae are about a day old. This ensures the production of robust, healthy flies.

Egg samples taken directly from a population cage contain too many held-back eggs for use in measuring mean hatching time. Taking adult flies directly from a cage for the purpose of egg collection is not very successful either. Among the flies in a cage there is always a substantial proportion of moribund individuals and it is extremely difficult to know how many cage flies to extract for purposes of egg collection. The number changes from day to day and by following this procedure one does not get a consistently dependable source of eggs and one is very likely to bleed the cage population and seriously interfere with its cycle.

POPULATION DIFFERENCES IN MEAN HATCHING TIME

Mean hatching time and its 95 per cent confidence interval for the five populations are shown in figure 1. It is obvious that these measurements distinguish OR and Sy from the other three populations and also separate I_1 from SySP. I_2 is intermediate between I_1 and SySP and its confidence interval overlaps those of the other two. The measurements were made in a laboratory having temperature control: mean 25°C ± approximately 1.5°. In lapsed time the means range from 18:46 (1,126 minutes for OR to 20:04 (1,204 minutes) for SySP. It is certainly true that some populations differ significantly in mean hatching time.

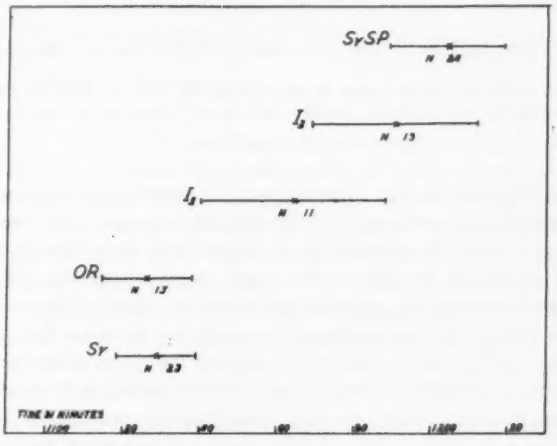

FIGURE 1. Mean hatching time in minutes with 95 per cent confidence intervals of five populations of *D. melanogaster* based on observations made from June 30 to August 13, 1959.

That the variances of these measurements result to a considerable extent from the relatively small fluctuations in temperature is strongly suggested by figure 2 in which means and confidence limits for OR and Sy are given for three different observation periods: 30/VI-13/VIII/59, 1-26/II/60 and 30/VI/59-26/II/60. Although the same temperature controls were operating, the mean temperature was slightly lower in the winter and the pattern of fluctuation was different. The mean hatching times for June, July and August were approximately two hours less than those for February. For the consolidated period, of course, means were intermediate.

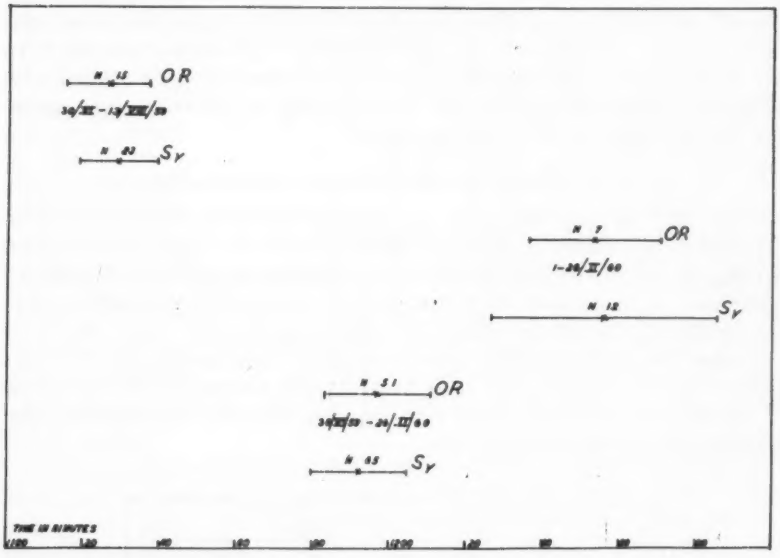

FIGURE 2. Mean hatching times in minutes of OR and Sy with 95 per cent confidence intervals for two different periods and for all observations accumulated over eight months.

To reduce the temperature fluctuations in these experiments much further would be technically awkward and financially ruinous. One can, however, do something toward the elimination of temperature and other environmental differences acting on the developing eggs by accumulating differences in hatching time between two populations in paired observations—those made at the same time. These measurements will not increase the precision of our knowledge of the mean, but by calculating the mean difference in hatching time for several paired observations and comparing this mean difference with its standard deviation, we can determine the probability that such a mean difference would be observed by chance if the mean hatching time of the two populations were equal, and we can state whether the mean difference between two populations is significant at any given level of probability.

Such calculations made on all the paired observations among the data represented in figure 1 produce the results given in table 1. At the 95 per cent confidence level those populations which were indistinguishable in figure 1 can now be separated. I_2 is distinguishable from both I_1 and SySP and the mean difference between even OR and Sy is greater than $t_{.95}$ times its standard deviation. The difference between the means is more constant than the means themselves; when the OR mean is high, the Sy mean tends to be correspondingly high. The standard deviation of the mean hatching time (5.02 for Sy and 5.05 for OR) is nearly twice the standard deviation of the mean difference (2.82). Environmental influences—of which temperature is probably the most important—produce fluctuations in the mean hatching time of

TABLE 1

Paired observations on mean hatching time
30/VI-13/VIII/59

Pair	n	$\bar{d}$	95 per cent confidence interval	$s_{\bar{d}}$	$\frac{\bar{d}}{s_{\bar{d}}}$	P
SySP-SY	20	69.6	± 9.658	4.63	15.032	$\ll .01$
SySP-OR	12	73.7	± 13.052	5.99	12.237	$\ll .01$
SySP-I_1	9	32.1	± 13.843	6.12	5.245	$\ll .01$
SySP-I_2	10	18.3	± 13.925	6.25	2.928	.01 .02
I_1-Sy	5	31.6	± 9.513	3.70	8.541	$\ll .01$
I_2-I_1	11	21.5	± 8.430	3.83	5.614	$\ll .01$
I_2-Sy	9	50.9	± 4.682	2.07	24.589	$\ll .01$
Sy-OR	14	6.36	± 6.049	2.82	2.255	.02 .05

Comparison of mean hatching times of five populations based on paired observations in eight population pairs giving number of observations (n), mean difference ($\bar{d}$), 95 per cent confidence interval, standard deviation of the mean difference ($s_{\bar{d}}$), the t value $\left(\frac{\bar{d}}{s_{\bar{d}}}\right)$, and the probability (P) of observing so great a mean difference if the two mean hatching times were equal.

both Sy and OR so that the confidence limits overlap to a considerable extent. But when the environmental influences are held constant, or nearly so, in paired observations, there is evidence that there is a real difference between the two populations in mean hatching time. This difference is extremely minute—a little more than six minutes out of more than 1,100 minutes of embryonic development, about 0.5 per cent—but it is sufficiently constant for 14 comparisons to tell us that by chance alone such a difference would occur only once in 20 repetitions of these observations.

CONSTANCY AND CHANGE IN THE DIFFERENCE BETWEEN Sy AND OR

In spite of the very considerable shifting of the mean hatching time of Sy and OR, the mean difference in paired observations proves to have been astonishingly constant—with an interesting and illuminating exception—over a period of eight months, from June 30, 1959, to February 26, 1960. Table 2

TABLE 2

Paired observations on mean hatching time
Sy–OR

Period	Dates	n	$\bar{d}$	95 per cent confidence interval	$s_{\bar{d}}$	$\frac{\bar{d}}{s_{\bar{d}}}$	P
I	30/VI–22/VIII/59	14	6.36	± 6.05	2.82	2.255	.02–.05
II	5/X–23/XI	18	0.33	± 7.19	3.42	0.096	> .90
	a 5–15/X	5	– 10.60	± 4.34	1.69	6.272	< .01
	b 19/X–5/XI	9	1.33	± 11.67	5.16	0.258	> .80
	c 17–23/XI	4	11.75	± 17.66	6.36	1.847	.10–.20
III	30/XI–3/XII	4	6.50	± 5.61	2.02	3.218	.02–.05
IV	15–26/II/60	7	6.71	± 4.87	2.06	3.257	.01–.02
V	29/II–31/III	17	– 6.59	± 4.20	1.99	3.312	< .01
VI	5–28/IV	15	– 0.73	± 2.90	1.36	0.537	.60 ±
VII	2–26/V	16	0.75	± 4.77	2.25	0.333	.60–.80
VIII	31/V–30/VI	19	– 2.74	± 3.85	1.84	1.489	.10–.20

Comparison of mean hatching times of OR and Sy by means of paired observations for eight different periods. For explanation of symbols see table 1.

gives the pertinent figures for these differences. After February 26 (Periods V–VIII) the mean difference shows some erratic shifting which will be discussed later. The constancy is apparent in the figures for the periods I, III and IV during which the mean difference was between six and seven minutes and the standard deviation of the mean was between two and three. For all three periods the difference is significant at the 95 per cent level.

For period II from October 5 to November 23, 1959, during which 18 paired observations were made, the results are different. The mean difference is only 0.33 minutes with a standard deviation of 3.42, more than ten times the difference itself. Such a mean difference would be expected to result from chance alone more than 90 per cent of the time. If we examine this aberrant period in greater detail, we find that it is not homogeneous. The 18 observations fall into three consecutive groups. The first five observations are all negative; the last four all positive; and the intervening nine are partly positive and partly negative. Table 2 gives the figures for the full period and for the three sub-periods (a, b and c).

Remembering that period II lies chronologically between periods I and III in both of which the mean difference was strikingly similar and very nearly what it was in period IV three months later, we have evidence that a persistent difference in mean hatching time between two populations was shifted so as to reverse its sign and that over a period of approximately a month it returned to its former magnitude and variability. During the period of return (IIb and c) its variability was higher than before or after.

Between periods I and II the two populations underwent an environmental change which must in some way have altered the developmental rates. On August 24 the temperature control in the laboratory, which had been running at 25°C ± 1.5°, broke down and remained out of operation until September 17, 24 days later. During this time the laboratory temperature was con-

stantly above 25°C and on most days rose to 28° or 29°. Bottle cultures which were not removed from the laboratory succumbed; the populations survived, evidently because evaporation from the medium in the cups kept the temperature in the cages below that of the ambient air. There were changes other than thermal, of course; judging from the odor emitted, the microflora must have changed, and the life-span of the individuals must have been shortened although precise data of this sort are completely lacking.

At 25° the generation time in a cage is about two weeks; at the higher temperature during the heat wave it must have been somewhat less. During the 24 days there must have been at least two generations. The first egg sample after the heat wave was taken September 21. The eggs tested to give the first measurement of mean hatching time for period II were laid by flies which developed from the September 21 sample. Measurements made on eggs laid by flies developing from a second egg sample taken September 28 all gave negative differences of mean hatching time and are included in sub-period IIa. The first positive difference was observed with eggs laid by flies grown from an egg sample of October 7, 21 days—well over a generation—after the restoration of temperature control.

In the face of these facts it is difficult to escape the conclusion that the change which occurred was genetic. Eggs laid by flies which had not and whose parents had not been subjected to the higher temperature show the change in developmental rate. A phenotypic change which persists in F_1 and F_2 is usually assumed to be genetic. If it were not genetic, but some sort of complex cytoplasmic adaptation or Dauer modification, the two populations reacted differently and this must have been the result of prior action of different genomes on the cytoplasm. The change may have occurred in only one population or in both. We know only that OR hatched later with respect to Sy. Sy may have speeded up while OR remained constant; both may have slowed down, but OR more; or finally, both may have speeded up, but Sy more. The fact remains that the two populations reacted differently to the same environmental change; the effects of this reaction persisted for at least two generations after the environment had been restored to its former state and the effects of the reaction then gradually disappeared over a period of two or three more generations.

WHAT HAPPENED AND HOW?

The change in phenotype which occurred—the shift in relative mean hatching times—could not have been the result of direct selection. The two populations were side by side and subject to what must have been extremely similar environments, but they were separate and closed—not in competition. The relative change must have resulted from selective processes acting independently in each population. Nor is it likely that there was selection within each population for developmental rate as such. Even if one's conception of a chromosome can accommodate loci bearing alleles which add or subtract 6.5 minutes of developmental time, why should the same environment reduce the percentage of plus alleles in one population and increase it

in the other? Actually the shift in developmental rate was so slight—a maximum of about one per cent if all the change occurred in one population—that it taxes the imagination to invent a reason for its adaptiveness.

Since whatever genetic change occurred must have been effected in two or three generations, changes in frequency must have involved whole chromosomes rather than individual alleles. There was no time for an elaborate and painstaking reassortment and elimination. Rather, flies carrying certain chromosome combinations left relatively more offspring under the new environment. This resulted in a generation with a genetic make-up somewhat different from that of its predecessor. And being different genetically, it was also somewhat different phenotypically. Part of the phenotypic change was an increased adaptiveness in the new environment, but there were also numerous slight phenotypic shifts which were quite accidental. They were there because they happened to be characteristic of the individuals carrying the chromosomes which had increased in frequency.

Both these populations, having existed as closed units for more than six years, must certainly have attained equilibrium and their genetic systems must have been well integrated. If two populations have differently integrated genetic systems, one should not be surprised to see them respond differently to the same environmental change. The chromosomes which are increased in frequency in one population because of some primary influence on phenotype will have a number of secondary influences which will alter the means and variances of many other characters; but in another population the constellation of secondary influences contained on those chromosomes increased in frequency for the same primary influence will be different, perhaps strikingly so.

There is good evidence that the two populations here observed do have different genetic systems. When they were selected for resistance to DDT, they differed in response. On numerous occasions differences in their behavior have been obvious. When they were subjected to the aerosol containing DDT, the OR flies rested on the bottom of the flask; the Sy continued to fly around. It was an easy matter to tell by simple observation which strain was being treated. When, a few years ago, Sy flies were used for some experiments in Dr. Bruce Wallace's laboratory at Cold Spring Harbor, all the assistants who handled them commented on their greater activity as compared with OR.

It is most likely that the shift in mean hatching time was a secondary change. Changes in chromosome frequency caused slight shifts in mean hatching time and, since the two genetic systems were different, the shifts were not precisely the same in the two populations; this changed their relative values.

The period of about two generations at the higher temperature was clearly not long enough for thorough-going reintegration of the genetic systems. In two generations substantial changes in chromosome frequency can occur but the crossing-over necessary for intrachromosomal readjustment would require a longer time. It is not surprising, therefore, that when the environment re-

verted to what it had been, the relative developmental rates drifted back to the original state. When a rapid shift from one mean to another takes place, it is very likely to be accompanied by an increase in variance. Here because of the effect of temperature fluctuations our measurement of the mean is so imprecise that we cannot detect a slight increase in variance. Nor can we measure the variance of the hatching distribution accurately enough to demonstrate a change. Luckily, the variance of the mean difference is more sensitive and in sub-periods IIb and c when the shift must have been occurring it is decidedly larger than in any other period.

Cases of rapid shifts of chromosome frequencies in laboratory populations are, of course, well known. Without question the first instances brought to the attention of drosophilists were not the result of planned experiments. We have no record of the first mutant culture lost as a result of wild-type contamination. Many experiments could be cited in which natural selection was shown to have altered chromosome frequencies drastically in two or three generations. Perhaps the most dramatic of these is that described by Carson (1958). The introduction of a single haploid set of chromosomes into a closed laboratory population was followed within two generations by an enormous increase in the frequency of the chromosomes introduced. In this case the environment was held constant and new genetic material was added, but the change was effected by the differential increase of one type of chromosome as compared with another. When this happens, it means that individuals carrying the waxing chromosome must be leaving more offspring than those carrying its waning homologue.

When working with marked chromosomes, one may calculate frequencies with precision. But in the case of chromosomes which contribute slightly more or less to a given phenotypic character, one can merely deduce changes in frequency from shifts in the mean of the character. One deduces further that the shifts in both mean and frequency must have resulted from some form of differential fecundity.

An example of a correlation between an increased variation in fecundity and a phenotypic change in the succeeding generation came to light during the work with the Sy strain on resistance to DDT. In several crosses between two lines of Sy which were being selected for resistance to DDT, an increase in resistance was observed between F_2 and F_3 even though there had been no exposure to the insecticide in any generation of the cross including P_1 (King, 1958). Some automatic process was changing chromosome frequencies between these two generations. It looked as though the change must result from differential fecundity among F_2 females. F_2 and F_2 females were placed singly in vials and counts were made of the number of adult offspring per female. There was a significant difference in variance of number of offspring between the two generations. s^2 F_2 494; s^2 F_1 306; F 1.6; $F_{.95}(198,100)$ 1.32.

In a selection program for high sterno-plural bristle number in Drosophila, Scossiroli (1954) discovered that departure from the mean for this character was correlated negatively with fecundity. Flies at the extremes of the dis-

tribution were more likely to be sterile or to have reduced fecundity than those in the middle. Similar results have been obtained by Bruce Wallace (personal communication). These observations are of great interest in connection with the increased variance in number of offspring of F_2 females as compared with the F_1 in the cross between the lines selected for resistance to DDT. In those crosses where the LD_{50} increased from F_2 to F_3, the variance of the F_2 mortality distribution was greater than it was for the F_1 or the F_3. The F_2 had a greater variance both in phenotypic character and in the number of offspring per female. Scossiroli's finding strongly suggests that these two distributions were not independent, that the extreme phenotypic deviants were the less prolific. If this reduction were symmetrical on both sides of the distribution, it would tend to leave the mean of the next generation unaltered. If, however, it were more pronounced in one tail than in the other, the mean would be shifted. This is apparently what did happen in the case of the resistance to DDT.

These processes are undoubtedly involved in the explanation of what happened in the shift of the developmental rates of OR and Sy and this explanation gives us insight into the process of evolutionary change. A population at equilibrium has a pattern of chromosome frequencies which tends to remain constant. These chromosomes, when paired at random, produce zygotes characterized by a developmental pattern that produces an array of adults of a given constellation of phenotypic distributions. Chromosome composition and frequency, developmental pattern, modal phenotype and means and variances of phenotypic characters are all interrelated and mutually adjusted to maximize adaptedness in the prevailing environment.

A change in environment will cause changes in the relative fecundity of certain genotypes and this will result in a shift in chromosome frequencies in the following generation. This slightly different array of chromosomes when paired at random will produce a somewhat different set of genotypes, a different array of developmental patterns and an altered set of adult phenotypes. Among these adults, those deviating most from the former set of means will be least fecund and this will tend to return the population to its former state. If the environmental change is well defined and persistent, the population will gradually work out a new equilibrium involving adjustments in the genetic material, the developmental pattern and the constellation of adult phenotypes. If the environmental change is transient, the population will display a strong resilience tending toward a return to its former equilibrium.

Selection always acts on the complex tripartite system—genetic material, developmental pattern and phenotypic array—because generation after generation each one actualizes itself in terms of the other in a never ending progression. The whole system is always involved because selection can act only on whole organisms each of which embodies the system. It is impossible to select alleles, developmental patterns or adult characters except as these exist in living individuals. The first reaction of a population to an environmental stimulus is a relatively crude one; the increase in frequency

of certain chromosomes at the expense of others. Many alleles will be shifted in frequency quite accidentally depending on how they happen by chance—random genetic drift—to be distributed among the increasing and decreasing chromosome types. What is thrown out or made more frequent by accident of association will influence the effect which the selective agent will have on the whole population complex. Hence two apparently identical populations may react in opposite senses to the same environmental change. This is what happened to OR and Sy; their relative developmental rates were reversed. A somewhat similar phenomenon has been described by Prevosti (1958) who subjected two strains of *D. subobscura* to selection for increased wing length. Both responded positively but both also responded with a change in the number of teeth in the sex comb, one with an increase, the other with a decrease.

Considered in this light, genetic drift becomes more than a meaningless game of chance. It is creative. Populations diverge non-adaptively by chance and this divergence is amplified by selection into diversity. Genetic drift is neither a competitor of nor spurious substitute for selection. It is its collaborator.

A population can adjust to an environmental change with finesse and precision only if the change persists for some generations. The sorting out of different alleles at different loci requires numerous generations for recombination to produce the more effective genotypes. But all such readjustment takes place within the limits of the system as a whole. Too great deviations from the developmental pattern will result in inviable embryos; too great variation from the modal phenotype is likely to be accompanied by reduced fecundity; too high a percentage of one type of chromosome is likely to produce embryos over-sensitive to environmental vagaries. The population gropes for improved adaptation but it keeps a tenacious grip on everything from its past that it can fit effectively into its present. This is genetic homeostasis.

To be adaptive a character must be integrated not merely into the modal adult phenotype, but into the genetic and epigenetic systems as well. Thus what may appear to the observer to be the same character may be part of an adaptive complex in one population and not in another. In our present material, at the higher temperature more rapid embryonic development was part of an adaptive complex in Sy, but not in OR. What may look to us as advantageous to the adult may cause so much disturbance in the genetic or developmental system that the population may not be able to afford it. Conversely, a trait that looks to the outsider like a weakness may be the inescapable result of the over-all system of the population. Its elimination would require so fundamental an overhauling of the population design that moves in this direction are more likely to produce extinction than improvement—to lead into an adaptive valley in Wright's terminology.

A spot on a butterfly's wing, the arrangement of pigment in the single hair of a mammal or the shape of a human skull are not independent items which can be retained or rejected individually like the pieces in a toy construction

set. They persist or disappear as they harmonize with or disrupt the total population system. A system of a given sort having been built up, the probability of its being basically altered may be so remote that the population may be irrevocably headed in a given direction of evolution toward expansion or extinction. This state of affairs has been termed orthogenesis. Or, a character which has long persisted in a line may quite suddenly disappear if the constant throwing of the genetic dice turns up a route to a more adaptive system in which the character is no longer de rigueur. When the results of this process are observed, they are more likely to be explained by assuming replacement of the population from without rather than readjustment from within.

Evolution is irreversible because the population system is so complex and its reintegration so intricate that the probability of retracing exactly any series of steps is close to zero. It is highly unlikely that our OR and Sy populations in December and February when they were behaving with respect to relative time of development as they had been in July and August, were exactly the same as before. Even though the period at high temperature had not lasted long enough to effect a thorough-going readjustment of the population system, it is very likely that when the chromosomes which had been reduced in frequency increased again toward their former level, the total genetic material of either population was not precisely the same as before. Within each population the chromosomes eliminated were not identical and when they were replaced by differential replication of survivors, the restitution was not a slavish copy of the original.

In fact since February 26, 1960, (table 2) OR and Sy have not shown the constant mean difference in hatching time which they did in the preceding eight months. For March (Period V) the figures show an exact reversal of the previous relation. For the following three months (Periods VI–VIII) the mean difference has been smaller and without significance at the 95 per cent level. Whether these changes have resulted from genetic shifts within the populations or from changes in the laboratory environment which influenced the measurements, it is impossible to say. For Periods VI–VIII there was a slightly different temperature regime which may account for the disappearance of the significant difference although there is no obvious reason why it should. If the reversal of the difference in Period V was the result of changes in laboratory environment or in technical manipulation, the experimenters are not conscious of any such alterations. *A priori* one should scarcely be surprised at fluctuations of 0.5 per cent in the relative developmental rates of two separate populations. One is much more justified in being amazed that for eight months the fluctuations were consistently less. That this consistency could have resulted from chance errors of measurement is fantastic.

SUMMARY

Precise measurements of the time between fertilization and hatching of the eggs of *Drosophila melanogaster* show that different populations have

characteristic means. Slight temperature differences produce very substantial differences in mean hatching time, but between any two populations the difference between the means is more constant than the means themselves. Two large, randomly breeding populations in three periods of sampling over a lapsed time of eight months gave a mean difference in hatching time of from six to seven minutes in a total of over 1,100. In all three sampling periods this difference was significant at the 95 per cent level. Between the first and second of these sampling periods the populations were subjected to 24 days of higher temperature. In another period of sampling beginning four days after the higher temperature ceased, the difference in mean hatching time was at first reversed and during the next six weeks it gradually shifted back to where it had been. It remained at this level for another three months. Evidence is given to show that these changes were genetic and resulted from selection. The meaning of these observations is discussed in terms of genetic, developmental and population systems and in relation to the mechanisms of evolution.

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X-RAY INDUCED SEX-LINKED RECESSIVE LETHALS AND VISIBLES IN *SCIARA COPROPHILA**

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The studies on x-ray induced mutations in Sciara presented in this paper are of interest in connection with two problems: (1) the apparent resistance to the mutagenic effects of irradiation which this genus has repeatedly displayed; and (2) the sensitivity of different stages of the germ cell cycle to irradiation.

Uniformly, the rate of visible mutation in Sciara has been found to be exceedingly low (see Metz, 1938a, and Crouse, 1949). Only 26 mutant characters have been found as a total in studies on five species extending over a number of years. A comparison of the rates of induced mutation in Drosophila and Sciara cannot be stated numerically, for no absolute basis of comparison is available. The chromosome history and mode of inheritance in Sciara are such that in monogenic species like *S. coprophila* an induced autosomal recessive cannot be detected prior to the F_4 generation and then the odds against its detection are at least 15:1 (Crouse, 1949). Theoretically, the mode of inheritance and reproduction should not interfere with the appearance or detection of sex-linked factors. Nevertheless, exclusive of the mutants recovered in the present experiments, only five sex-linked factors have been found in *S. coprophila*. Although efficient technics such as attached—X's and C1B are *not* available in Sciara, the following study on sex-linked recessive lethal and visible mutations was possible by virtue of the very low frequency of crossing-over between sex chromosomes in the female germ line (see Crouse, 1943).

The problem of differential sensitivity to irradiation of different stages of the cell cycle is a large and complex one and no attempt will be made in this paper to go into it in detail. It is clear, however, that conflicting results may be obtained depending on the manner in which experiments are conducted (see Auerbach, 1954) and depending on the criteria of sensitivity chosen (see Bozeman and Metz, 1949). A primary question, still unanswered, is whether a given genetic locus is as likely to mutate at one stage of the cell cycle as at another. To answer this question the test organism should possess a number of features: it should be a form which is "known" genetically (good genetic maps) and cytologically, one in which mutants and multiple allelic series can be obtained, and finally a form in which the germ cells develop synchronously. Drosophila possesses all these features except the last one. In Sciara, on the other hand, both the male and female

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germ cells develop synchronously, but there is a dearth of genetic markers and no multiple alleles. Thus, Sciara can be used to advantage only for comparative studies on genetic effects of a less specific nature, namely, the induced frequency of sex-linked lethals or visibles.

In the experiments presented here the mutation rates induced in mature sperm (adult males) are compared to those induced in the immature adult oöcyte (prophase of first meiotic division). In both types of cell two sex chromosomes were present at the time of irradiation: two identical (sister halves) X's in the case of the sperm and in the case of the oöcyte an X and an X′. In both series of experiments only one of the two sex chromosomes from a given irradiated cell was tested for induced mutations: in the case of irradiated oöcytes this was by virtue of the fact that only F_1 females of XX constitution were selected for breeding, the XX′ females being discarded (see outline of experiments presented below under Materials and Methods); in the case of irradiated sperm it was by virtue of the fact that although two identical X's are included in the sperm nucleus of Sciara, one is eliminated from the embryonic germ line of all the progeny. Hence, the mutation rates obtained in the two series of experiments are exactly comparable.

MATERIALS AND METHODS

Use was made of two sex-linked factors and their wild type alleles: swollen, a recessive located on the X chromosome, and Wavy, a dominant located on X′. In this species sex of progeny is controlled by the sex chromosome constitution of the mother (see Metz, 1938b): thus, XX′ females give rise to families of daughters and are referred to as female-producers, whereas XX females give rise to families of sons and are termed male-producers. Females heterozygous for Wavy and for swollen (XX′) bred to wild type males are thus expected to yield daughters, half of wild phenotype and half Wavy. All of the F_1 daughters are heterozygous for swollen; the non-Wavy ones, which are XX in constitution, should produce sons of two kinds—wild type and swollen—in approximately equal numbers, provided, of course, that neither of the maternal X chromosomes carries a recessive lethal or a visible mutation.

The plan of the x-ray experiments, then, was as follows: (1) For the treatment of mature sperm wild type males were irradiated and bred to XX′ females heterozygous for Wavy and for swollen. The F_1 daughters were bred singly to males from stock and the progeny examined microscopically. If a recessive lethal had been induced on the irradiated X, no wild type sons should appear among the progeny, only sons of swollen phenotype. The presence of a few wild type sons in an F_2 culture could be explained theoretically in one of two ways: either a semilethal had been induced by the irradiation; or, the wild type flies were the result of crossing-over between a lethal factor and the swollen locus. Because it was impossible to distinguish experimentally between these two alternatives, no F_2 culture was scored as a recessive lethal if there were any wild type individuals in the

culture. Furthermore only F_2 cultures containing a minimum of ten flies were included in the study. The control lethal rate was derived from the number of F_2 cultures which showed the reverse condition, that is, zero males of swollen phenotype and a minimum of ten wild type individuals. (2) For the treatment of oöcytes female-producers (XX′) heterozygous for Wavy were irradiated and bred to swollen males. The F_1 non-Wavy daughters were in turn bred singly to stock males and their progeny (sons) examined microscopically. Any F_2 culture which yielded zero wild type and a minimum of ten swollen males was scored as a lethal. Again, the control lethal rate was based on the number of F_2 cultures showing the reverse condition. Sex-linked visibles in both types of experiment could be detected by the presence of the mutant phenotype in approximately 50 per cent of the F_2 progeny. Whether the mutant was induced or spontaneous (control) could be determined in a given experiment by the phenotype of the non-mutant F_2 sibs.

The x-ray irradiation was done at the Department of Biology, Johns Hopkins University, under the supervision of Dr. Timothy Merz. The flies were exposed in gelatin capsules at a distance of exactly 23.0 cm. from the tube window. The dosage rate in all experiments was approximately 1060 r/min., the voltage and current exactly 250 KV and 30 ma. Careful correction for temperature and barometric pressure was made in all cases. The experiments were conducted during the period October, 1958–July, 1959, at Goucher College, Baltimore, Maryland.

I wish to thank Dr. Merz and Professor Carl Swanson for the assistance rendered in connection with the x-ray exposures.

RESULTS

Presented in tables 1 and 2 are the results from the experiments on irradiated males (sperm). Although the data are not extensive, they clearly

TABLE 1

Recessive sex-linked lethals induced in sperm

Experiment No.	Dose	No. F_1 ♀♀ tested	No. F_2 cultures	No. lethals	F_2 count
M1X2	2000 r	28	23	1	11 sw:0+
M2X2	2000 r	69	47	0	
M3X2	2000 r	98	77	1	23 sw:0+
M1X3	3000 r	40	24	1	33 sw:0+
M2X3	3000 r	46	15	1	11 sw:0+
M3X3	3000 r	103	60	1	26 sw:0+
M1X4	4000 r	15	10	0	
M2X4	4000 r	23	5	0	
M3X4	4000 r	248	186	7	24 sw:0+
					43 sw:0+
					50 sw:0+
					13 sw:0+
					27 sw:0+
					17 sw:0+
					50 sw:0+
M3X4	Control	(670)	(447)	1	0 sw:33+

TABLE 2

Summary of recessive sex-linked lethals induced in sperm

Dose	No. F_1 ♀♀ tested	No. F_2 cultures N	No. lethals n	Mutation rate n/N ± S.E.
2000 r	195	147	2	.0136 ± .0096
3000 r	189	99	3	.0303 ± .0172
4000 r	287	201	7	.0348 ± .0129
Control	670	447	1	.0022 ± .0022

indicate that recessive sex-linked lethals arise at an appreciable rate in this genus and that the rate is approximately linear with dose. The standard error of the rate n/N was computed as follows: SE $n/N = \sqrt{\frac{n/N(1 - n/N)}{N}}$.

Two sex-linked recessive visibles were obtained in these experiments, one in M1X2 and one in M3X3. Both were wing characters; in the case of the former the F_2 progeny count was 24 swollen: ten mutant; in the latter the F_2 count was 15 swollen: 17 mutant. The former mutant proved to be associated with a reciprocal X-translocation, the latter with no gross rearrangement. The latter has been retained in culture and designated "petite."

TABLE 3

Mutations induced in irradiated oöcytes

Experiment No.	Dose	No. F_1 ♀♀ tested	No. F_2 cultures N	No. lethals n	F_2 count	Rate n/N ± S.E.
1FX4	4000 r	259	137	6	45 sw : 0+ 28 sw : 0+ 13 sw : 0+ 26 sw : 0+ 44 sw : 0+ 12 sw : 0+	.0437 ± .0174
Control		259	137	0		.0000

Presented in table 3 are the data on the x-rayed oöcytes. The females at the time of irradiation were freshly-emerged and none was older than seven hours; hence the oöcyte nuclei were in prophase of the first meiotic division. Because the oöcyte chromosomes at this stage have proved to be so refractory to x-ray induced aberrations (Bozeman and Metz, 1949), only the maximum dosage of 4000 r was administered. Surprisingly, it yielded a sex-linked lethal rate comparable to that induced in mature sperm (0.0348 versus 0.0437). This difference is not significant; a 2×2 table (with Yates' correction) gives a χ^2 value of < 0.01 and thus a P value of 90–95 per cent. No sex-linked visibles were obtained from the irradiated oöcytes.

DISCUSSION

The experiments by Spencer and Stern (1948) on x-ray induced sex-linked recessive lethals in Drosophila sperm are far more extensive than the ones

presented here on Sciara. Nevertheless, it is of interest to compare the mutation rates obtained. For dosages of 2000 r, 3000 r, and 4000 r the Drosophila rates were .0471, .0650, and .0987 respectively, while those for Sciara were .0136, .0303, and .0348. It should be remembered that the technic used for detecting the lethals in Sciara was considerably less efficient than the Muller-5 method employed by Spencer and Stern; as pointed out above, only those F_2 cultures were scored as lethals in the Sciara experiments in which there was no crossing-over between the lethal and the locus for swollen.

The sex-linked visible rate (2/447 = .0044) induced in mature sperm, although based on meager data, seems high in view of the dearth of visibles obtained previously in this genus. It is interesting to note that both of the mutants recovered were wing characters as have been all the sex-linked factors obtained previously in *S. coprophila*. This result bears out the suggestion (Crouse, 1949) that the biochemical pathways in Sciara result in a more restricted range of phenotypic variability than is the case for Drosophila.

Using chromosomal aberrations as a criterion of oöcyte sensitivity to x-rays in *S. ocellaris*, Bozeman and Metz (1949) found early first meiotic prophase to be completely insensitive (zero per cent of F_1 larvae showed aberrations) and first anaphase to be highly sensitive (40–50 per cent of F_1 larvae showed aberrations) to the same dosage of irradiation (1100 r). Using the same criterion of sensitivity Metz and Boche (1939) found that approximately 27 per cent of F_1 larvae derived from irradiated sperm (5000 r) showed chromosomal aberrations. It is very interesting therefore that in the present study the sex-linked recessive lethal rate induced in sperm did not differ significantly from the rate induced in oöcytes at first meiotic prophase. There seems to be no correlation between the induction of chromosomal aberrations and the induction of recessive lethals.

SUMMARY

A study was made of the sex-linked recessive lethal and visible mutations induced by x-rays in the sperm and oöcytes (first meiotic prophase) of *S. coprophila*. For sperm irradiated at 2000 r, 3000 r, and 4000 r the lethal rates obtained were 0.0136, 0.0303, and 0.0348 respectively. Sperm and oöcytes irradiated at 4000 r gave approximately the same lethal rate, 0.0348 versus 0.0437. Two sex-linked recessives were recovered from the experiments on irradiated sperm (2/447 = 0.0044), an appreciable visible rate in view of the fact that only five sex-linked traits have been recovered heretofore in this species.

On the basis of the experimental data the following interpretations have been made: (1) Sciara is *not* resistant to the mutagenic effects of x-rays. The low yield of visible mutations obtained repeatedly in this genus can be attributed to a number of factors, including the unusual mode of inheritance and sex determination found in these flies. (2) There is no correlation between the induction of chromosomal aberrations and the induction of sex-linked recessive lethals. When the latter are used as a criterion of sensi-

tivity to x-rays, the response of sperm and oöcyte (first meiotic prophase) is not significantly different. When chromosomal aberrations are used as a measure of sensitivity, however, the sperm are found to be highly sensitive whereas the oöcytes are completely insensitive (zero aberrations).

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THE CONTRIBUTION OF HETEROZYGOSITY AT CERTAIN GENE LOCI TO FITNESS OF LABORATORY POPULATIONS OF *DROSOPHILA MELANOGASTER*

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Classical evolutionary theory suggests that genotypes producing characters which favor the chances of their owner surviving and reproducing tend to be perpetuated in a mendelian population. Genotypes producing characters less favorable to the organism's survival in that environment tend to be eliminated from the population. Thus a mendelian population would be expected to have a high degree of homozygosity for the genes controlling favorable characters. The majority of spontaneously arising mutations, having a high probability of being harmful to the given organism's adjustment to its environment at the time of occurrence, would be eliminated. Only those very few mutations which happened to be beneficial to the organism at the time of occurrence would be retained and incorporated into the genotype of the population.

In more recent years a large body of evidence has been accumulated which shows that wild mendelian populations are not highly homozygous but instead display an array of genotypes representing much heterozygosity (see Dobzhansky, 1951). The evolutionary significance of this widespread heterozygosity has occupied the attention of population geneticists in recent years.

Methods have been developed for keeping populations of Drosophila in the laboratory under controlled environmental conditions which simulate the action of a rigorous natural environment in putting the flies under natural selection in order that evolutionary changes in the genetic structure of the population will ensue and can be observed. L'Heritier and Tessier (1937) followed the fate of various mutant genes which were introduced into populations of flies kept in large cages with controlled amounts of food. They found that, rather than being eliminated, certain mutant genes tended to become balanced in the population at a definite level (see also Tessier, 1943, 1947 a, 1947 b). Other work with modifications of their method produced further evidence for persistent genetic variability in laboratory populations of *Drosophila melanogaster* (Buzzati-Traverso, 1955; Kalmus, 1945; Kerr and Wright, 1954; Ludwin, 1951; Merrell and Underhill, 1956; Reed and Reed, 1948, 1950).

It has been theorized that balanced polymorphism, both of the genic type seen in some of the above experiments and the chromosomal type found in various wild populations, gives adaptive value by helping to buffer the population against extremes of various environmental conditions such as temperatures. The superiority of the heterozygotes under such conditions is

sufficient to keep the polymorphic condition balanced in the population, even at the expense of the inevitable production of certain homozygous individuals which will be less fit than the population as a whole (Dobzhansky, 1951; Dobzhansky and Wallace, 1953; Da Cunha, Burla, and Dobzhansky, 1950; Wallace, 1954). Buzzati-Traverso (1955) has suggested that heterozygosity for groups of polygenes as well as for single genes may serve as the selective basis of increased adaptive value, and that natural selection acting over a period of time may bring about visible or measurable changes in the individual flies that parallels increased fitness of the organism to its environment. This increased fitness might also show up as an increase in the total biomass produced by a certain population in a certain environment (Buzzati-Traverso, 1955).

Carson (1958) has modified the population-cage technique in such a way that he is able to check the phenotypic composition of the hatch of the population's offspring every two days, and weigh the entire population once a week, while maintaining his populations under standardized conditions with controlled amounts of food. He produced balanced polymorphism in an inbred laboratory stock of *Drosophila melanogaster* homozygous for the third chromosome recessive mutants sepia (se), spineless (ss), and rough (ro) by adding to this population a single male carrying one haploid set of wild-type Oregon-R autosomes and one haploid set of those from the se ss ro stock. After the addition of this one set of wild-type autosomes to the experimental population, the frequency of the mutant alleles rapidly declined and then stabilized, producing balanced polymorphism in the population (Susman and Carson, 1958). The fitness of the three populations (the inbred se ss ro, the polymorphic one just described, and a wild-type one of Oregon-R flies only) was compared by keeping the populations under natural selection for 15 generations and using population biomass as a criterion of fitness. The fitness of the new polymorphic population [to be designated hereafter as se ss ro (Oregon)] surpassed not only that of the inbred population but also that of the wild-type population. Simple heterosis in the polymorphic population was suggested as an explanation for the increased fitness (Carson, 1958).

The present experiment was designed to determine whether the heterosis observed by Carson was exclusively due to heterozygosity at the sepia, spineless, and rough loci or whether it was also due to heterozygosity at other, unmarked loci, possibly including heterozygosity for polygene groups. This question was to be investigated by deriving from Carson's polymorphic se ss ro (Oregon) population, which had displayed the heterosis, a new population which would be phenotypically entirely sepia, spineless, and rough, but which, being a direct descendant of the heterotic population, would presumably contain the unknown genetic factors carried on the Oregon-R chromosomes whose presence might also account for the heterosis. The performance of this new population could then be compared with the original inbred sepia spineless rough population and also with the polymorphic heterotic population. With heterozygosity at the sepia, spineless, and rough

loci removed in the new experimental population, the contribution of heterozygosity at these loci to population fitness could be assessed. The data show that if any contributions to fitness are made by heterozygosity at these loci, the amount is very small.

MATERIALS AND METHODS

The flies used in this experiment are described in table 1. Populations 1 and 3 were descendants of the polymorphic populations in which Carson had found increased fitness. Phenotypically they were largely wild-type with some mutant individuals (largely single mutants with some double mutants and a very few triple mutants) appearing regularly in the hatch. These mutants appearing in the hatches were used to establish Populations 2 and 4, which thus consisted of the mutant offspring of Populations 1 and 3 respectively, plus their own mutant offspring, any wild-type offspring appearing in the hatches of Populations 2 and 4 being placed in Populations 1 and 3. Figures 1 and 2 show that mutants appeared regularly in Populations 1 and 3 under the conditions of natural selection to be described below, despite the artificial removal of the mutants as they showed up in the hatch.

These four populations were maintained in the population vials under conditions of natural selection from January 30 to June 5, 1959. These vials contained a measured amount of medium made according to the recipe of Carson (1958). Just before use each vial had a strip of paper towel placed

TABLE 1

Origin and composition of the populations of *Drosophila melanogaster* described in this paper

Population number	Origin	Composition of base population	Autosome sets introduced	Operations in this experiment	
				Treatment prior to testing	Final combination at testing
1	E-1a, Carson, 1961	se ss ro	Oregon-R	artificial removal of mutant phenotypes	se ss ro (Oregon-R) control
2	E-1a, Carson, 1961	se ss ro	Oregon-R	mutants fixed by reselection	se ss ro (reselected) experimental
3	E-9a, Carson, 1961)	Oregon-R	se ss ro	artificial removal of mutant phenotypes	Oregon-R (se ss ro) control
4	E-9a, Carson, 1961	Oregon-R	se ss ro	mutants fixed by reselection	se ss ro (reselected) experimental
5	C-1, Carson, 1961	se ss ro	none	none	se ss ro (original) control
6	C-1, Carson, 1961	se ss ro	none	none	se ss ro (original) control

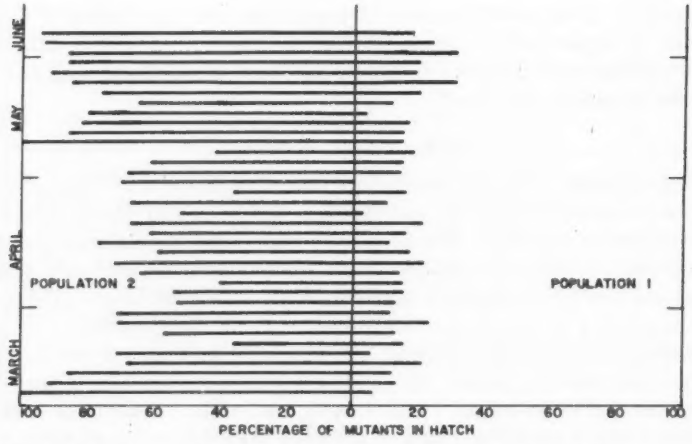

FIGURE 1. Per cent of mutant phenotypes hatching in Population 1, se ss ro (Oregon), and in Population 2, which was originally derived from the mutants of the hatch from No. 1. The populations were under natural selection for the period of time shown here, while at the same time artificial selection was being applied to the hatch (see text).

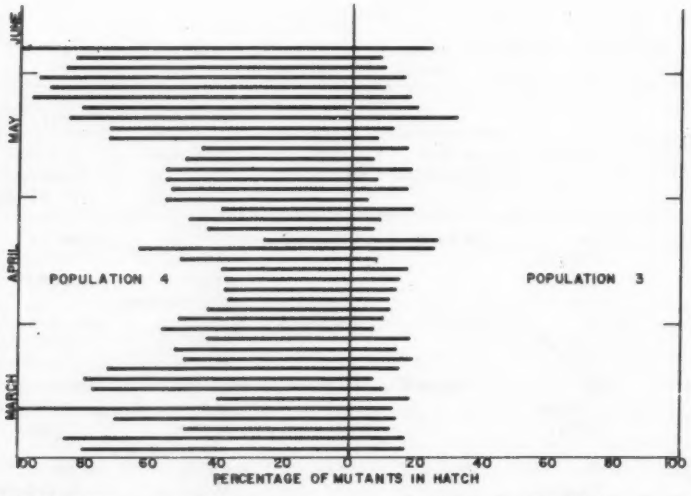

FIGURE 2. Per cent of mutant phenotypes hatching in Population 3, Oregon (se ss ro), and in Population 4, which was originally derived from the mutants of the hatch of Population 3. The populations were under natural selection for the period of time shown here, while at the same time artificial selection was being applied to the hatch (see text).

in it to absorb water, and the food, a weighed amount of Fleischmann's dry yeast (13 milligrams of yeast three times a week for each population during this phase of the experiment) was added to the vial with a drop of distilled water. When the population of flies was installed in the vial, a "super vial" consisting of a plastic tube the same diameter as the vial with a strip of cardboard in it was attached to the vial and its open end plugged with cotton, this arrangement giving the flies additional space. When the population was transferred to a fresh yeasted vial, the vial from which it was removed was plugged with cotton and saved. This vial contained the eggs laid by the flies during their sojourn in it; flies hatched out from it for as long as five weeks after the parent population had been removed from it. Each time the population was transferred to a new vial, the hatch from all of these accumulated vials was collected, examined phenotypically, and added to the appropriate population as already described. During the course of the entire experiment, the temperature of the laboratory was 25°C., ±1°, except for brief periods.

The reselection process designed to produce populations in which sepia, spineless, and rough were fixed in homozygous recessive form was carried out as follows from June 5 to September 28, 1959. Theoretically, these new populations were to differ from the populations already described by the replacement of wild alleles at the sepia, spineless, and rough loci by the mutant alleles, with the rest of the genetic structure of the population left intact as far as possible. Therefore the new populations could not be descended from a single triple recessive male and female, but must be descended from a large number of flies, so that the background genetic variability in the ancestral population would be largely preserved in the new population. Therefore Populations 2 and 4 were removed from the population vials and allowed to proliferate in stock bottles for several weeks. From the large number of flies thus obtained, single mutant males were selected and crossed with virgin female mutants of the same type. From the progeny of these crosses, double recessive males of the three possible types (sepia spineless, sepia rough, and spineless rough) were selected and mated with similar double recessive virgin females. From the progeny of these crosses triple recessive males were obtained and mated to triple recessive virgin females. The progeny of the triple recessives obtained in this manner from Population 2 were allowed to proliferate, then divided and set up in population vials to form replicate populations 2A and 2B. Likewise, replicate populations 4A and 4B were formed from the triple recessives descended from Population 4. Table 2 shows the numbers and phenotypes of flies used in these crosses. It is believed that a genetic bottleneck and the effect of random drift have been avoided by this procedure and that the reselected populations 2A and 2B, 4A and 4B have retained the background genetic variability of the populations from which they were derived. Control populations 1 and 3 were set up by taking at random from Populations 1 and 3, which had also been allowed to proliferate in stock bottles, the same number of flies as were placed in the population vials to start the reselected populations.

TABLE 2

The establishment of Populations 2A and 2B, 4A and 4B by reselection of se ss ro

Population 2		se ss ro	se ss	se ro	ss ro	se ss ro	Total
Step 1: P August, 1959	Virgin females	8 3 30					41
	Males	circa 50					50
							91
Step 2: F_1 September, 1959	Virgin females		0	8	20		28
	Males		2	22	19		43
							71
Step 3: F_2	Virgin females					circa 35	35
	Males					circa 35	35
							70
Step 4:	Splitting of hatch from Step 3 into 2 replicate populations, 2A and 2B						
Population 4		**se ss ro**	**se ss**	**se ro**	**ss ro**	**se ss ro**	**Total**
Step 1: P August, 1959	Virgin females	4 5 25					34
	Males	circa 50					50
							84
Step 2: F_1 September, 1959	Virgin females		13	4	9		26
	Males		18 plus 28 more	2	6		26 28
							80
Step 3: F_2	Virgin females					circa 35	35
	Males					circa 35	35
							70
Step 4:	Splitting of hatch from Step 3 into 2 replicate populations, 4A and 4B						

Populations 1, 2A, 2B, 3, 4A, 4B, 5 and 6 (the latter two, as shown in table 1, being the inbred se ss ro populations) were placed in population vials September 28, 1959, and maintained according to Carson's methods (Carson, 1958). The vials were changed four times per week, and the flies given ten milligrams of yeast at each change, a total of 40 milligrams per week. The hatch was counted and added to the population at each vial change. Once a week each entire population was weighed and counted. The hatch was examined to ascertain that Populations 2A, 2B, 4A, 4B, 5, and 6 consisted only of sepia spineless rough flies, while Populations 1 and 3 were ascertained to continue producing the various possible mutants among the wild-type flies. Populations 1, 2A, 2B, 5, and 6 were terminated February 19, 1960, having completed a run of 16 weeks after equilibrium of population size had been attained.

RESULTS

Figures 3 and 4 and table 3 show the results of the comparison of fitness of the reselected monomorphic populations 2A and 2B with the sepia spine-

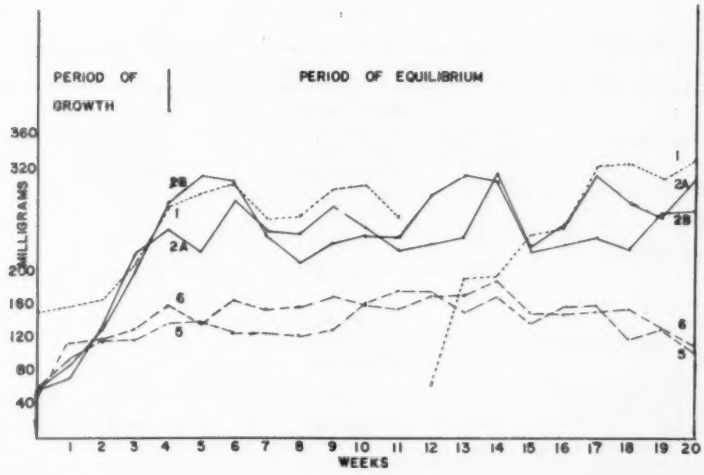

FIGURE 3. Performance of five populations of *Drosophila melanogaster* measured by milligrams wet weight of the total population over a period of 16 weeks at equilibrium. ····· is the polymorphic control Population 1; ——— are the reselected monomorphic Populations 2A and 2B; – – – – are the monomorphic control Populations 5 and 6. The break in the dotted line indicates an accidental loss of part of Population 1.

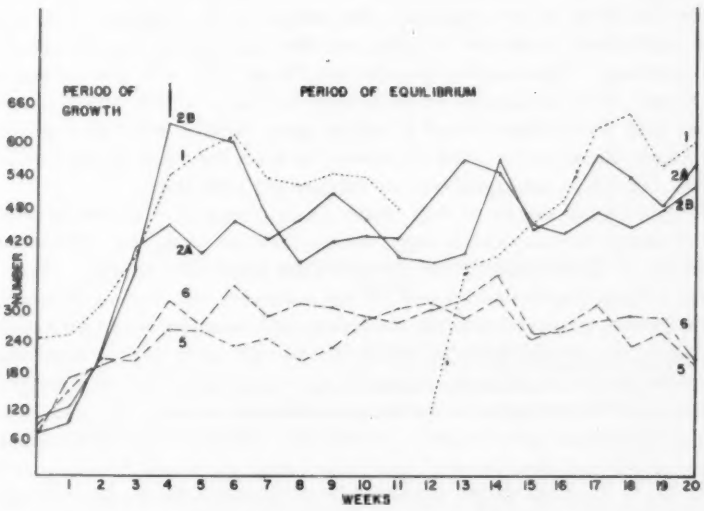

FIGURE 4. Performance of five populations of *Drosophila melanogaster* measured by number of flies in the population over a period of 16 weeks at equilibrium. ····· is the polymorphic control Population 1; ——— are the reselected monomorphic Populations 2A and 2B; – – – – are the inbred monomorphic control Populations 5 and 6. The break in the dotted line indicates an accidental loss of part of Population 1.

TABLE 3

Mean weekly weights and sizes of Experimental and Control Populations of *Drosophila melanogaster*

Population	Weeks at equilibrium	Mean weight of population per week (milligrams)	Standard error	Mean number of flies in population per week	Standard error
Controls					
No. 5 se ss ro	16	142.41	± 5.94	261.2	± 8.80
No. 6 se ss ro	16	155.47	± 4.62	290.6	± 8.69
No. 1 se ss ro (Oregon)	8	283.50	± 6.39	543.4	± 12.81
No. 3 Oregon (se ss ro)	24	275.40	± 5.40	551.4	± 11.67
Experimentals					
No. 2A se ss ro (Oregon) reselected	16	261.94	± 7.49	468.2	± 15.04
No. 2B se ss ro (Oregon) reselected	16	261.59	± 8.01	492.2	± 17.79

less rough controls (Populations 5 and 6) and the control Population 1 which had shown the marked superiority in Dr. Carson's work. The weight of the flies is given in milligrams wet weight which includes the dead flies present in the vial when it was emptied. The counts of the numbers of flies were made immediately after the weighing and the dead flies were eliminated before counting. Experimental populations 2A and 2B, with mean weights of 261.9 and 261.6 milligrams respectively, consistently run about 1.7 times higher than Populations 5 and 6, whose mean weights are 142.4 and 155.5 milligrams respectively. The difference between the mean weights of Populations 5 and 6 is not significant at the five per cent level.

When the mean weight of Population 2A is compared with that of Population 1 using the comparable eight weeks data for each, the difference is found to be significant at the five per cent level ($P = 0.004$). When the means of both Population 2A and 2B are compared with that of Population 1 using the full 16 weeks data for the former and the eight available weeks for the latter, the results are just within the five per cent level of significance ($P = 0.04$ and $P = 0.049$ respectively). A comparison of the mean weights of Population 6 and Population 2A over the 16 week period gives a highly significant difference ($P < 0.0001$). These data indicate a definite superiority of the reselected experimental populations over the inbred homozygous controls and a possible slight superiority, of questionable statistical significance, of the polymorphic control population over the experimental monomorphic populations.

Figures 5 and 6 show the results of the comparison of reselected populations 4A and 4B with Population 3. Flies with long bristles (flies not spine-

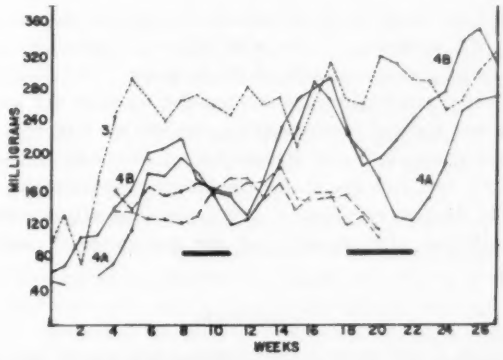

FIGURE 5. Performance of five populations of *Drosophila melanogaster* measured by milligrams wet weight of the total population over a period of 27 weeks. ····· is the polymorphic control Population 3; ——— are reselected monomorphic Populations 4A and 4B; – – – – are inbred monomorphic control Populations 5 and 6. The horizontal bars indicate periods at which sizeable portions of the hatches of Populations 4A and 4B were eliminated (see text).

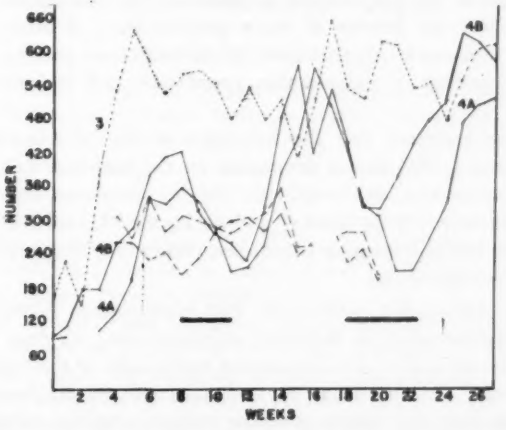

FIGURE 6. Performance of five populations of *Drosophila melanogaster* measured by number of flies in the population over a period of 27 weeks. ····· is the polymorphic control Population 3; ——— are reselected monomorphic Populations 4A and 4B; – – – – are monomorphic inbred control Populations 5 and 6. The horizontal bars indicate periods at which sizeable portions of the hatches of Populations 4A and 4B were eliminated (see text).

less, although sepia and rough) appeared at various times in Populations 4A and 4B, despite rigorous attempts to eliminate them. It was assumed that these flies represented contaminations in the populations, although Buzzati-Traverso (1959) has suggested that the appearance of bristles may be due to a number of polygenic factors acting to modify spineless, rather than to the presence of the wild allele of spineless. Crosses made with the

non-spineless flies found in Populations 4A and 4B did not conclusively prove whether the presence of the wild allele of spineless or some other factor was causing the appearance of the bristles. The elimination of flies from these affected populations so altered the sizes of the populations that the data were not treated statistically. Inspection suggests results compatible with the comparisons of Populations 1, 2A, and 2B: significant difference between the fitness of the reselected monomorphic experimental populations and the inbred controls, and a possible slight superiority, probably not significant statistically, of the polymorphic controls over the experimentals.

DISCUSSION

The comparison of the various experimental and control populations shows that the monomorphic reselected populations have a fitness level significantly greater than the original, inbred, phenotypically similar sepia spineless rough populations. Fitness levels in these new populations are very close to the polymorphic populations from which they were derived. It would appear, therefore, that the replacement of the wild alleles of sepia, spineless, and rough in the polymorphic populations by their mutant alleles does not greatly lower the fitness of these populations. A slight lowering, of questionable statistical significance, is nevertheless evident in one of the comparisons between a polymorphic population and its monomorphic derivative.

Thus it would appear that the heterosis of the polymorphic population found by Carson (1958) is not dependent on the presence of heterozygosity at the sepia, spineless, and rough loci. Sepia, spineless, and rough mutants were balanced in his populations, and as figures 1 and 2 show, remained balanced when the polymorphic populations used in this experiment were exposed to natural selection.

Dobzhansky (1951) has pointed out that heterozygote superiority is requisite to the maintenance of balanced polymorphism, whereas the data just presented indicate a lack of heterozygote superiority at the sepia, spineless, and rough loci. This apparent contradiction can be resolved by assuming that the heterozygosity which actually carries adaptive value in the polymorphic populations and also in the reselected monomorphic populations is not at the loci of these marker oligogenes but is found in blocks of polygenes (or possibly to some extent in unknown oligogenes) which are phenotypically invisible but which in the polymorphic populations occur in close linkage with sepia, spineless, and rough. Heterozygote superiority could thus be largely maintained even after the fixation of the sepia, spineless, and rough mutants in the experimental populations by the artificial reselection process. The hypothesis of Buzzati-Traverso (1955) that polygene groups play a large part in fitness is compatible with these results.

SUMMARY

Comparison of the fitness of three types of populations of *Drosophila melanogaster* has been made. These are (1) inbred monomorphic controls,

(2) heterotic polymorphic populations, and (3) monomorphic populations reselected from the polymorphic ones. These reselected populations have the genetic background of the polymorphic populations but the phenotypes of the monomorphic controls. Fitness was compared by measuring the weights and numerical sizes of the populations when maintained under controlled environmental conditions where natural selection operates. The reselected populations have been found to closely parallel the original heterotic polymorphic populations in fitness. Their performance is only slightly below these heterotic populations but very significantly above the inbred monomorphic controls. These data indicate that the heterosis found originally by Carson in the polymorphic populations is largely the result of heterozygosity for unknown genetic factors, perhaps polygene blocks, and only due in small degree to heterozygosity at the loci of sepia, spineless, and rough, the mutant marker genes in the monomorphic populations. Single gene heterosis apparently contributes little.

ACKNOWLEDGMENTS

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X-RAY-INDUCED CHROMOSOME ABERRATIONS AND REPRODUCTIVE DEATH IN MAMMALIAN CELLS

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Recent years have seen the development of important technical advances that have made it possible to investigate radiation-induced damage in mammalian cells quantitatively. Specifically, it has been found practical to grow clonal colonies from large numbers of single mammalian cells *in vitro* (Puck and Marcus, 1955; Puck *et al.*, 1956). Also, the use of colchicine, hypotonic saline prefixation treatments, and tissue culture have made it possible to obtain good cytological preparations of mammalian chromosomes (Hsu and Pomerat, 1953). Because of the current interest in the damaging effects of radiation to man, x-ray survival curves of tissue-cultured human cells have been studied (Puck and Marcus, 1956; Puck *et al.*, 1957). For the same reason, x-ray induction of chromosome aberrations in human cells *in vitro* has been investigated (Bender, 1957, 1960; Puck, 1958; Chu and Giles, 1959).

We believe that the amount of accumulated data on radiation damage to mammalian, especially human, cells *in vitro* warrants an examination of the relation between these data and the previously acquired data on invertebrate and plant cells. In particular, we will consider two questions: the relative sensitivity of mammalian cells, and the relation between chromosome aberration induction and mammalian cell killing.

CHROMOSOME ABERRATIONS

Over the years, a large body of information has been acquired showing that radiation can induce visible chromosome aberrations. The work of Karl Sax and his coworkers formed the basis for a quantitative theory of visible aberration production (see Giles, 1954). Breaks induced in a chromosome can either remain open or rejoin in one of two different ways. They may either restitute (rejoin in the original configuration) or reunite with other broken ends to form complex rearrangements. If they undergo rearrangement or remain open, visible chromosome aberrations will be found at the next metaphase. These nuclear changes can be arbitrarily classified as being chromosome aberrations or chromatid aberrations, depending on whether the break occurs before or after duplication of the chromosomes. Most of the quantitative work on mammalian cells has been done on chromatid aberrations, which are the first to appear after irradiation. Chromosome aberrations have also been observed, however. The aberrations induced can be further subdivided into a group that is largely dependent on breakage alone,

*Operated by Union Carbide Corporation for the United States Atomic Energy Commission.

such as chromatid deletions and isochromatid breaks, and a group that depends on the production (and reuniting) of at least two different breaks, such as chromatid exchanges. The former class exhibits mainly linear or 1-hit kinetics, indicating that the probability that irradiation of the cell will break a chromosome is directly proportional to the dose. The latter class exhibits nonlinear (2-hit) kinetics approaching a dose square relation, as might be expected, since the probability of getting two independent breaks that can interact will be the product of the probabilities of getting the individual breaks.

The kinetics of aberration production

The kinetics of these types of aberrations has been extensively studied by many plant cytologists (see Lea, 1955). The mammalian data have now been shown to follow a similar pattern with single break (1-hit) aberrations increasing approximately linearly with x-ray dose, and two-break aberrations seeming to increase as the square of the dose (Bender, 1957, 1960; Chu and Giles, 1959). It is difficult to show statistically, however, that the mammalian curve for two-break aberrations is truly nonlinear. The reason for this is that, even with the newer cytological techniques, it is still far harder to obtain and score a large number of mammalian cells than plant cells, as in *Tradescantia* and *Vicia* that have a small number of much larger chromosomes. The similarities observed between plant and animal chromosomes are, however, so striking in all other respects that it seems likely that the apparent 2-hit kinetics as observed is real.

The yield of two-break aberrations per cell, Y, may be expressed

$$Y = a + bD + cD^2$$

(Catcheside *et al.*, 1946), where D is the dose of radiation, and a, b, and c are constants. This equation takes into account both the spontaneous frequency and the fact that occasionally both breaks of a two-break aberration may be formed by a single ionizing particle. With some material the linear term is quite large but it is negligible in others. In either case, it should be noted that there is no threshold effect for a 2-hit phenomenon. Even when the linear term of the equation is zero, there is a finite probability proportional to the square of the dose that a 2-hit aberration will be induced no matter how small the dose.

The sensitivity of the chromosomes

It has long been known that, in plants, the sensitivity of cells to radiation varies over a wide range and is influenced by such factors as hydration, stage of cell division, and whether or not the cell is actively metabolizing (see Swanson, 1957). Although the kinetics of chromosome aberration production may be the same in plant and mammalian cells, the sensitivities of the two kinds of cells might be expected to be quite different. Nevertheless, there is a similarity in the sensitivity of mammalian cells in culture and of *Tradescantia* microspores that indicates that mammalian chromosomes

do not exhibit a greater sensitivity than has been observed for the plant chromosomes. Lea (1955, table 64) compiled data on the sensitivity of *Tradescantia* chromosomes. His measure of sensitivity, called the coefficient of production of aberrations, was simply the coefficient a in the equation $Y = aD$ for 1-hit aberrations, and the coefficient b in the simplified expression $Y = bD^2$ for the two-break aberrations, where Y is the yield and D is the dose. We have fitted data from our experiments to the equations by the least-squares method in order to estimate the values of these coefficients. (We are greatly indebted to Dr. A. W. Kimball both for the statistical computations contained in this paper and for many helpful suggestions during its preparation.) The cells used in these computations were all from samples in their first postirradiation division (see Bender, 1960). The

TABLE 1

Coefficients of x-ray-induced aberration and break production calculated from data (Bender, 1957, 1960) on mammalian cells and data (Lea, 1955) on *Tradescantia* microspores.

Tissue	Chromatid breaks/cell per r	Isochromatid breaks/cell per r	Exchanges/cell per r^2
	($\times 10^2$)	($\times 10^2$)	($\times 10^3$)
Tradescantia microspores	0.725 ± 0.08	0.271 ± 0.02	1.81 ± 0.21
Human epithelioid cells	0.066 ± 0.10	0.246 ± 0.16	0.55 ± 0.35
Monkey epithelioid cells	0.043 ± 0.21	0.237 ± 0.49	0.38 ± 0.34
Chinese hamster "fibroblasts"	0.047 ± 0.16	0.409 ± 0.20	0.45 ± 0.29

values for chromatid aberrations are presented in table 1, together with the comparable values from Lea's data on *Tradescantia*. It will be seen that there is a large discrepancy in the values for chromatid deletions. Our values are less than one-tenth those for *Tradescantia*. They are also lower than the values calculated by other authors from their data on mammalian cells (Puck, 1958; Wakonig and Ford, 1960) for mammalian cells *in vitro*. At least part of the discrepancy is attributable to scoring (as breaks) aberrations that have been called achromatic lesions. Since there is some controversy about the nature of achromatic lesions (Revell, 1959), we have excluded them from our totals. The differences in sensitivity between the mammalian and the plant cells are in any case in the direction of a *lower* sensitivity of mammalian chromosomes.

We should like to point out that the seemingly low values for the coefficients of aberration production reported here should in no wise be interpreted as being a measure of the amount of genetic damage inflicted on the cell. These coefficients refer simply to the final aberrations that are observable at metaphase. The large majority of the breaks induced restitute, that is, return to the original configuration without giving rise to any observable aberration. Thus the large number of primary breaks is not reflected in the values of these coefficients. Although it has been suspected

that the primary breaks that restitute can also be sources of genetic damage, the relation of observable aberrations to primary breaks and to detrimental point mutations is complex and still obscure (see Muller, 1954).

The distribution of breaks

Another striking similarity of the effects of radiation in inducing aberrations in mammalian and plant cells is that the breaks and aberrations are found to be distributed according to the Poisson formula $e^{-m} \cdot m^r/r!$, where m is the mean number of aberrations observed in the experiment and r is the number 0, 1, 2, 3, . . . , of aberrations per cell. Lea (table 56) accumulated data from several sources for plant (*Tradescantia*) and animal (*Chortophaga*) cells and showed that, for all types of chromatid aberrations, the distributions observed fit the Poisson distribution. This has since been found true

TABLE 2

Tests for Poisson distribution of x-ray-induced chromatid aberrations in mammalian cells in tissue culture

χ^2 test made by comparing the observed mean and variance (Cochran, 1954). The number of degrees of freedom is large, and $t = \sqrt{2\chi^2} - \sqrt{2(\text{d.f.}) - 1}$ is used to calculate p.

Material and x-ray dose	Aberration	Aberrations per cell						Mean	Variance	t	p
		0	1	2	3	4	5				
Human epithelioid cells, 50 r	Chromatid	247	9	0	0	0	0	0.0352	0.0340	−0.35	0.64
	Isochromatid	226	25	5	0	0	0	0.1367	0.1577	1.70	0.05
	Exchange	252	4	0	0	0	0	0.0156	0.0154	−0.09	0.54
Monkey epithelioid cells, 100 r	Chromatid	498	22	4	1	0	0	0.0629	0.0857	5.44	<0.001
	Isochromatid	401	101	21	2	0	0	0.2838	0.3067	1.29	0.10
	Exchange	508	16	1	0	0	0	0.0343	0.0369	1.26	0.10
Chinese hamster "fibroblasts," 200 r	Chromatid	95	14	1	0	0	1	0.1892	0.3547	5.51	<0.001
	Isochromatid	61	37	6	6	1	0	0.6396	0.7780	1.56	0.06
	Exchange	99	9	2	1	0	0	0.1441	0.2153	3.33	<0.001

for all types of aberrations with the single exception of chromosome (not chromatid) exchanges (Atwood and Wolff, *in* Wolff, 1959). Table 2 gives the data for the distribution of aberrations per cell in mammalian cells. The data for human cells fit the Poisson distribution, as do most of the data for the monkey and hamster. In the three cases where the data do not fit the Poisson distribution, the aberrations are overdispersed, that is, there are too many cells containing multiple aberrations. There is also a tendency toward such overdispersion in the rest of the data, since the variance is usually larger than the mean. In any case, these calculations indicate that, when cells are sampled at the first metaphase after irradiation, a random sample of the cells containing damage is obtained. There is certainly no selection against heavily damaged cells; they appear in the final sample.

The fate of aberrations

The similarities reported, both quantitative and qualitative, in the induction of chromosome aberrations in mammalian cells and plant cells lead us to believe that, where the data are lacking in mammalian material, it is proper to generalize from the extensive information accumulated about the effects of radiation on plant chromosomes. It is, for instance, necessary to score cells that are at the first division after irradiation. The reason for this is that many of the aberrations induced are cell lethal after mitosis and will not undergo more than one division. Others may undergo a few divisions but not many, so that if the scoring is done at later divisions, we cannot be certain how many of the aberrations have been lost. In onion root tips, the loss is fairly rapid and progressive (Sax, 1941); by the time the root is 20 mm. long, only normal cells are left in it because of this selection against damaged cells. As an example of this type of loss, we might visualize a terminal deletion or simple chromatid break in which a portion of the chromatid is no longer attached to its centromere. The genes on this piece of chromosome will not move to the poles during anaphase and will not be incorporated into the nuclei of the daughter cells; therefore, there will be a loss of the genes on this particular chromosome. If the piece is extensive or if the genes contained on it are of vital importance to the cell, the cell that is deficient for this portion of the chromosome will die after the first cell division. This aberration would not show up in cells scored at subsequent metaphases, and there would be an apparent decrease in the numbers of aberrations induced by the radiation. If the genes on the deleted portion of the chromatid do not have vital importance, so that the cell might live even in their absence, the daughter cell that gets the centromeric portion of the deleted chromatid may go on dividing. The daughter chromosome derived from the deleted chromatid will have a shorter arm because of the deletion. In particularly favorable material, if the deletion is large (which seems rather improbable since the larger the piece the more vital we might expect it to be), we might notice a difference between its length and the length of the homologous chromosome. In routine scoring of aberrations and especially with difficult material like human cells, however, this type of effect would usually not be noticeable after the first division. The expected diminution in the numbers of aberrations observed at later divisions has been noted (Bender, 1957, 1960; Chu and Giles, 1959; Wakonig and Ford, 1960) in mammalian cells. This diminution of 1-hit aberrations should not be confused with restitution, which occurs before the first postirradiation division.

The "mean break dose"

The coefficients of aberration production for human epithelioid cells given in table 1 can be used to calculate the dose of x-rays that will produce an average of one scorable break per cell per roentgen. The assumptions are made that the chromatid and isochromatid deletions increase linearly with

dose ($Y = \beta_{cd}D$ or $Y = \beta_{iso}D$), that exchanges increase as the square of the dose ($Y = \beta_{excb}D^2$), and that the events leading to the aberrations are mutually independent. The mean number of aberrations produced is then $Y_{aber} = (\beta_{cd} + \beta_{iso})D + \beta_{excb}D^2$. Since each exchange involves two breaks, the number of breaks produced is then $Y_{br} = (\beta_{cd} + \beta_{iso})D + 2\beta_{excb}D^2$. Setting the latter expression equal to 1 and solving for D yields 190 r as the dose that will produce an average of one visible break per cell. Making the same calculation for aberrations indicates that a dose of 220 r will produce an average of one visible aberration per cell and is thus what has been termed (Puck, 1958) the "mean chromosome-damaging dose" for these cells.

Even though "fibroblasts" are more sensitive than epithelioid cells, we believe that the sensitivity estimates arrived at by Puck are in error, in that he assumes that at the "mean chromosome-damaging dose," the number of cells with two or more breaks would be "vanishingly small." The highest dose where no 2-hit aberrations were seen was found to be at 40–60 r, which was then taken to be the mean break, or 37 per cent dose. Since the breaks are distributed according to the Poisson formula, however, at the mean break dose, e^{-1}, or 37 per cent of the cells would be expected to have no breaks, $1 \cdot e^{-1}$ (the second term of the Poisson expansion), or 37 per cent to have one break, and 26 per cent (certainly not a "vanishingly small number") to have two or more breaks.

Chromosome sensitivity in vivo

It has been shown that "fibroblast-like cells" in culture have much higher aberration rates than epithelioid cells (Puck, 1958; Bender, 1960).

In the study of induced and spontaneous aberration rates in human "fibroblast-like cells" (Puck, 1958), the scored cells were fixed at times ranging from a few minutes to seven days after irradiation (probably, then, for up to at least seven divisions after irradiation). Few if any of the cells that were fixed at later times after irradiation could be expected to be in the first postirradiation division; cells in this division are the ones that must be scored because observable aberrations are lost in subsequent divisions. The observed breakage rates were erratic, with fewer aberrations observed at high doses than at low doses; the rate at 45 r was about 0.04, at 75 r about 0.02, and at 150 r about 0.01 break per cell per r. Some of the variability is doubtless caused by the low numbers of cells scored in some of the dose groups (three cells in the 10-r group; 20 cells in the 45-r group), but much must be attributable to the presence of various proportions of first and subsequent division cells in the various samples. The method of scoring used leads to an *underestimate* of the aberration rate; it may be concluded that the breakage rate for human "fibroblast-like cells" calculated from Puck's data is too low.

The question then arises whether the sensitivity of either type of cell *in vitro* is a good measure of the sensitivity of human cells *in vivo*. The "fibroblast" not only has a higher induced aberration rate than epithelioid cells but is even more unusual in that it has an extremely high spontaneous aberration rate. Thus human epithelioid cells (Bender, 1957, 1960) have a spontaneous aberration rate of about 0.01 breaks per cell, whereas human "fibroblast-like cells" have a rate between about 0.22 breaks per cell (Puck, 1958), and 0.1 breaks per cell (Chu and Giles, personal communication) including, as might be expected, two-break aberrations. To determine which, if either, cell type *in vitro* is representative of rapidly dividing cells

in vivo, additional experiments were performed with monkey and Chinese hamster bone marrow cells *in vivo*, and epithelioid and "fibroblast" cells *in vitro* (Bender, 1958, 1960, and unpublished data). The coefficients of aberration production are about the same for all these cells, but the spontaneous aberration frequencies are much lower *in vivo* than *in vitro*. In fact, out of 261 monkey and 281 Chinese hamster control bone marrow cells, no aberrations of any kind were found. It therefore seems that epithelioid cells provide a more realistic measure of the sensitivity of human somatic cells *in vivo* but even the epithelioid cells in culture have an abnormally high spontaneous breakage rate.

MAMMALIAN CELL SURVIVAL CURVES

The mechanism of x-ray-induced reproductive death of single mammalian cells, as measured by the cells' inability to undergo mitosis enough times to form macroscopic colonies, has been examined extensively by Puck and his coworkers (Puck and Marcus, 1956; Puck *et al.*, 1957). In general, the published x-ray survival curves of the various cell lines investigated are of the simple sigmoidal form

$$S = [1 - (1 - e^{-kD})^n]^m \quad (1)$$ *

where S is the surviving fraction, D is x-ray dose, m is the number of sites of damage, and n (the "hit number") is the number of each of the m kinds of sites that must be inactivated to prevent the survival of the cell.

To fix our ideas on the possible meaning of such a mathematical model, let us consider a genetic model (Tobias, 1952) in which a triploid cell has $a, b, c \ldots m$ genes, each present in triplicate. The probability that, for instance, gene a will not be hit and inactivated is e^{-kD}, and the probability that it will be hit at least once is $1 - e^{-kD}$. The probability that all three copies of gene a will be hit and inactivated is $(1 - e^{-kD})^3$ and the probability that this will not occur, and that gene a will "survive" is $1 - (1 - e^{-kD})^3$. However, for the organism to survive, all its m genes must survive; the cumulative probability for this is given by the equation $S = [1 - (1 - e^{-kD})^3]^m$.

That mammalian cell survival curves may be fitted by expression (1) has been confirmed by several authors (Bases, 1959; Elkind and Sutton, 1959). In the published studies, m is arbitrarily set equal to 1, k (or $1/D_0$) calculated from the slope of the linear portion of the curve, and n determined from the intercept (n^m) of the extrapolated linear portion (see Atwood and Norman, 1949). Puck and his collaborators find that, with m set at 1, n ranges from 2 for the hypotetraploid HeLa S3 line to "somewhere between 1 and 2" for diploid human "fibroblast-like cells," with D_0 values of 96 and 50 r, respectively, from which k may be calculated to be 0.01 and 0.02. The observation of extrapolation numbers "close to 2" and of high values of k has

*Puck has also used the form $N/N_0 = 1 - (1 - e^{-D/D_0})^n$, where $N/N_0 = S$, and $1/D_0 = k$; D_0, the "mean lethal dose," is thus given directly.

made it attractive to conclude that x-ray killing of mammalian cells is caused largely by the production of chromosome aberrations of the multiple-hit type (Puck, 1958), and numerous arguments have been presented in favor of this hypothesis (Puck, 1959a, b).

"Survival" from 2-hit aberrations

Since one of the main reasons that the chromosomes have been singled out as the site of the damage that results in x-ray killing of mammalian cells is that the hit number is 2 when m is arbitrarily set at 1, it seems important to see whether the fraction of an irradiated population of cells that do not have visible 2-hit chromosome aberrations (corresponding to the survivors) exhibit the same survival kinetics. Although the data on mammalian cells are still insufficient to allow such an analysis, such information is available for plant cells. Figure 1 shows the fit of experimental data (Atwood and Wolff, unpublished; from unpublished data of A. Conger and S. Wolff) from x-rayed *Tradescantia* microspores not having a 2-hit chromosome aberration, to the theoretical curve:

$$Y = [1 - (1 - e^{-0.0016D})^2]^{4.1}.$$

Since 2-hit exchanges were scored, n was known to be exactly 2. The least-squares method then gave values of $m = 4.1$ and $k = 0.0016$, from which the curve was drawn. The fit of the experimental points to the theoretical curve is extremely good. Thus actual scoring of 2-hit chromosome aberrations with a hit number of 2 shows that m is not equal to 1. The fact that n^m, which is the parameter measured with cell survival curves, was 2 does not mean that killing parallels (and might be attributed to) two-break chromosome aberration formation, for in such cases n^m does not equal 2^1 but, at least in *Tradescantia* microspores, $2^{4.1}$.

The relation between chromosome aberrations and cell killing

In addition, the theoretical relation of chromosome aberrations to mammalian cell killing is complex and obscure. Both 1- and 2-hit aberrations might be expected to cause the same sort of genetic anomaly that might result in failure of the cell to form a visible colony. Thus both 1-hit isochromatid breaks and 2-hit chromatid exchanges can lead to anaphase bridge formation; of the isochromatid breaks, all that have proximal sister union will form bridges, whereas one-half the exchanges can be expected to form bridges. Both isochromatid deletions and exchanges lead to chromosome deficiencies in the daughter cells resulting from the first postirradiation division. All isochromatid deletions are likely to produce deficiencies in both daughter cells. Asymmetrical chromatid exchanges lead to one normal and one deficient daughter cell, if no bridge is formed. Symmetrical exchanges lead to duplication-deficient daughter cells one-half of the time and two nondeficient daughter cells the other half. It is not known whether anaphase bridge formation is of itself lethal in mammalian cells. If the bridge breaks and daughter cells are formed, however, both will be deficient.

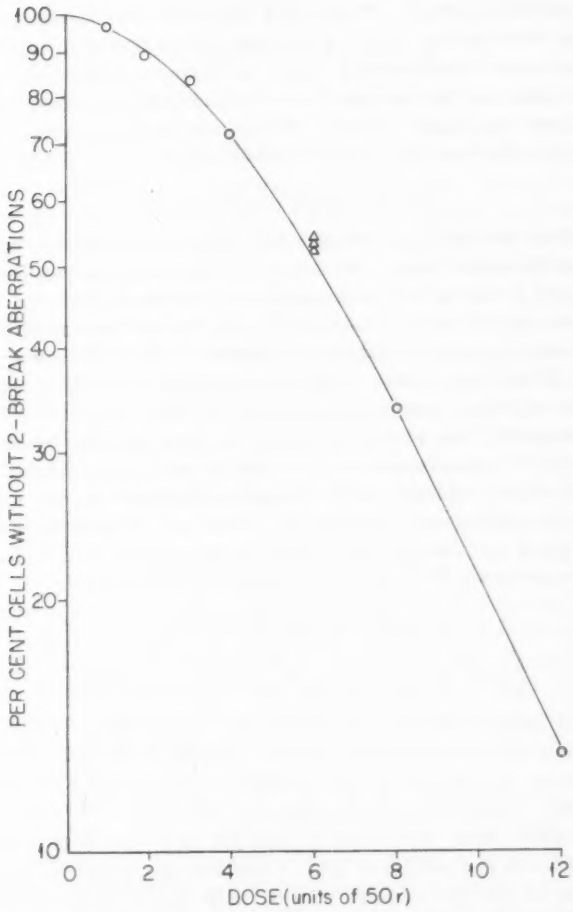

FIGURE 1. Fit of data on numbers of x-rayed *Tradescantia* microspores having no aberrations to the theoretical curve:

$$S = [1 - (1 - e^{-kD})^2]^m,$$

where $k = 0.0016 \pm 0.00016$, and $m = 4.1$. From unpublished calculations of Atwood and Wolff; ⊙ points are data of A. D. Conger (unpublished), △ points are data of Wolff (unpublished).

Chromatid deletions, of course, lead to deficiency in one of the daughter cells. If even one of the daughter cells is normal a colony can be formed. The less-common, more-complicated aberrations, such as rings and dicentrics, have even more-complex fates.

Although deficiencies for a portion of a chromosome are frequently lethal in diploid cells, the mammalian cells used in most survival studies are not diploid, but rather hypotetraploid, with normal variation in chromosome num-

ber (Chu and Giles, 1958). These cells obviously can tolerate gross duplications and deficiencies. Also, it has been shown that even diploid "fibroblasts" can survive deficiencies (Ford and Yerganian, 1958). It is therefore not at all clear that the deficiencies resulting from chromosome aberration production are necessarily lethal. If they are, however, both 1- and 2-hit aberrations would cause the same sort of lethality.

The extrapolation number, n^m

Although an extrapolation number (n^m) equal to 2 would not be expected if reproductive death were, indeed, caused by 2-hit chromosome aberration production, it is instructive to examine the shapes of mammalian cell survival curves more closely. Some authors (Puck and Marcus, 1956; Puck *et al.*, 1957) have reported extrapolation numbers only in the range of 1 to 2, but others (Elkind and Sutton, 1959) have reported much higher numbers.

Experimental x-ray survival curves may be fitted by the simple expression [equation (1)], but such a procedure involves the assumption that only one kind of killing process exists. As was pointed out by Atwood and Norman (1949), if any evidence exists that this assumption is not valid, equation (1) cannot adequately describe the situation. A mathematical expression that gives the survival that might be expected if cell killing can be caused independently by 1-hit and multiple-hit processes is

$$\prod_i [1 - (1 - e^{-k_i D})^{n_i}], \text{ or}$$

$$S = [e^{-kD}]\,[1 - (1 - e^{-k_2 D})^{n_1}]\,[1 - (1 - e^{-k_3 D})^{n_2}] \ldots \tag{2}$$

where each term represents the chance of survival from 1-hit, n_1-hit . . . events. As a first approximation, the first two terms of (2) will suffice and, by using these, we may see whether mammalian cell survival curves actually fit equation (2) better than they do equation (1).

Unfortunately, it is impossible to test the fit of the more complete expression (2) with the published data, since they are not given in numerical form. Some of our own survival curves, to be published elsewhere, show, however, a much better fit to the first two terms of equation (2) than to equation (1), although when fitted with equation (1) they are in general agreement with the published data. Thus, in experiments with a hypotetraploid clonal line (7A6) from a culture of normal human heart muscle, where survivals were measured down to 0.0005, we get $k = 0.008 \pm 0.0003$ and $n = 1.188 \pm 0.071$ (this was an unusually low n; they are usually nearer 2) if we use equation (1), but we get $k_1 = 0.0076 \pm 0.0004$, $k_2 = 0.0069 \pm 0.0006$, and $n_1 = 46.0 \pm 19.7$ if we use the first two terms of equation (2). When the data are fitted with equation (1), the residual sum of squares is 3.600; when they are fitted to equation (2) the residual sum of squares is only 0.300.

A model of a chromosome aberration killing process may be made that is similar to that used earlier to test data on *Tradescantia* microspores not having 2-hit aberrations. Such an equation, which takes into account sur-

vival from both 1- and 2-hit lethal aberrations, may be written

$$S = e^{-k_1 D}[1 - (1 - e^{-k_2 D})^2]^m . \quad (3)$$

This equation is similar to equation (2), except that n of the second term becomes 2 and m, the number of sites at which breaks that can participate in an aberration can occur, is inserted. The best fit of our data with equation (3) is $k_1 = 0.0056$, $k_2 = 0.00001$, and $m = 33{,}109$. The residual sum of squares in this case is again 0.300. It must be pointed out, however, that this model is such that a wide range of m and k_2 values will fit almost equally well. For this reason the calculation of standard errors is not valid. We attach no particular significance to the values of the parameters k, m, and n that we have obtained. We wish to point out, however, that our data may be fitted with a number of reasonable models, several of which show a much better fit to the experimental points than the simple model used as evidence for a 2-hit chromosome aberration mechanism of radiation killing. Also, although there is a wide range of possible error, the parameters obtained by fitting equation (3) are in disagreement with the idea that mammalian cell radiation killing is caused exclusively by chromosome aberration production.

The mechanism of cell killing

Since the relation of chromosomal rearrangements to cell killing is obscure, we should not ignore other possible mechanisms of damage. Among the possible mechanisms that we might suspect is inactivation of the centriole, which would prevent division and subsequent colony formation from irradiated cells. Such centriole inactivation has been postulated to occur in irradiated sea urchin sperm (Rustad, 1959) and in yeast (Lindegren *et al.*, 1959).

Another possible mechanism is inhibition of cell division after nucleolar inactivation. For instance, experiments with microbeam ultraviolet irradiations of grasshopper neuroblasts (Gaulden, 1958) have shown that these cells fail to divide if even one of their nucleoli is irradiated. Such an inactivation of a single mammalian cell in culture would, if irreversible, preclude colony formation.

Similarly, human cells that have been heavily irradiated *in vitro* enter mitosis in a relatively normal fashion but fail to complete mitosis, *not* because of the difficulties that might be expected to result from chromosome aberrations, but because they are unable, for some reason, to complete metaphase and enter anaphase (Harrington, 1960).

It therefore seems likely that, in cell lines that yield either high extrapolation numbers or multiprocess kinetics, and probably in all mammalian cell lines, x-ray-induced cell killing can result from damage to a number of sites, certainly including the chromosomes, but also including other targets (such as the nucleolus and the centrioles) and probably also some cytoplasmic target or targets whose identity remains unknown.

CONCLUSIONS AND SUMMARY

We found the kinetics of x-ray induction of chromosome aberrations in mammalian cells similar to that studied extensively in plants. In particular, the shapes of dose curves for one- and for two-break aberrations are similar in both types of material, as are the distributions per cell of the various types of aberration. It may be concluded that (1) there is no threshold dose for aberrations and (2) random samples of damage are obtained from cells sampled at the first postirradiation metaphase.

The dose required to produce one break per cell in irradiated human diploid epithelioid cells *in vitro* has been calculated to be about 190 r. This dose is similar to the dose required to produce one break per cell in mammalian cells *in vivo*, but much higher than the value for "fibroblasts" *in vitro*.

Studies of the x-ray survival kinetics of a hypotetraploid human cell line similar to that used in other studies have shown that the survival of these cells better fits a compound curve containing both single- and multiple-hit components than a simple sigmoidal curve.

Data on the numbers of cells not having x-ray-induced 2-hit chromosome aberrations show that these cells do not fit the quantitative pattern required by the hypothesis that x-ray-induced cell killing is caused mainly by the production of visible 2-hit chromosome aberrations. Although it is reasonable to assume that these aberrations cause a proportion of such killing, their relation to killing is obscure, and certainly other targets, both cytoplasmic and nuclear, should be taken into consideration.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

CHROMOSOME BREAKAGE IN STRUCTURAL HETEROZYGOTES

Natural populations of many Dipteran species are polymorphic for series of structural arrangements differing by simple or complex inversions. Several investigators have been struck by the non-random distribution of such "natural" inversions over the genome. In *Drosophila pseudoobscura, D. persimilis* and *D. athabasca* (Dobzhansky, 1944; Novitski, 1946) the structural polymorphism exhibits a pronounced preference for one member of the chromosome set; moreover, the inversion break points are clustered in certain sections of this chromosome. In other types, like *D. robusta* (Carson, 1958) *D. willistoni* (da Cunha, Burla and Dobzhansky, 1950) *D. subobscura* (Goldschmidt, 1956; Kunze-Mühl and Müller, 1958) and some Chironomids (Beermann, 1955; Rothfels and Fairlie, 1957) and Simuliids (Basrur, 1959), the polymorphic variation involves most or all of the chromosomes, but again, certain portions of the map stand out as distinctly favored break regions.

Over ten years ago Novitski (1946) discussed the mechanisms which might be responsible for this non-random localization of naturally occurring inversions. Since the early radiation experiments (Helfer, 1941) had failed to show any agreement between the distributions of induced and natural break points, Novitski hesitates to ascribe the clustering of the latter to the intrinsic break sensitivity of certain chromosome regions.[1] He gives only brief consideration to the role of natural selections, which might have picked a particular assortment of inversions out of a random choice. He proposes, instead, that the different break events occurring in any population may be causally related. According to this hypothesis the presence of any inversion in a structural heterozygote will favor the production of similar inversions with break points closely adjacent to those of the first. The structurally heterozygous bivalent is assumed to present the loop configuration, which is so well known in salivary chromosomes. In such a loop the inversion break points are marked by shorter or longer asynaptic regions, which may result from the change of direction in the pairing association of the partners. Novitski proposes that the twisted asynaptic portions of the homologues are subject to mechanical stress and therefore tend to break. Moreover, two breaks which have arisen in this manner are likely to produce a new inversion on healing, because of the close proximity of the two "wounds" in the loop configuration.

[1] Again, in a recent experiment Mainx and collaborators found that radiation induced breaks in *D. subobscura* although non-randomly localized, were clustered in different regions than those of the naturally occurring inversions (Mainx, F., 1958, V Rassegna Intern. Elettronica Nucleare Roma: 569).

It is difficult to accept this attractive theory in its original version, since mechanical stress is not generally recognized as a cause of chromosome breakage (excepting the extreme case of the dicentric bridge chromosome). In view of the high stability of the "balanced" laboratory strains of Drosophila, in which each individual is a structural heterozygote, the twisting strain as such can hardly be considered a very active force in producing additional chromosome aberrations. Under experimental conditions, at all events, the agents responsible for chromosome breakage are ionizing radiations and radiomimetic chemicals.

However, Novitski's hypothesis requires only a slight modification in order to gain contact with the radiogenetic theory of structural changes. Instead of assuming that the mechanical stress at the exchange points of synaptic direction in the inversion loop gives rise to chromosome breakage, we propose that this stress interferes with normal chromosome repair. The evidence from various lines of investigation suggests that many of the primary break events remain undetected owing to normal restitution (Russell, Russell, and Kelly, 1958; Puck, 1960; Wolff, 1960). If in the inversion heterozygote the chance of repair is diminished for hits occurring in the "twisted" sections, such breaks are more likely to participate in the production of aberrations. The regions adjacent to the break points of the original inversion will thus mimic an "induced break-sensitivity." The sensitive stage might be meiotic prophase in both sexes, although the details of synapsis in higher Dipteran males are still obscure, while the somatic pairing characterizing Dipteran mitosis might render the gonia, too, susceptible to the effect.

It is only in its modified form that the hypothesis lends itself to an experimental test. A pilot experiment has been carried out on a small scale. *Drosophila melanogaster* males heterozygous for the inversions III/L, III/R Payne and for the structurally standard homologue, which carried the dominant marker Lyra (Ly), were irradiated on the first day after emergence with an x-ray dose of 5000 ± 100 r (therapeutic x-ray source, 200 KV, filters 1 mm Cu, 1 mm Al). During five days of "storage" the males were provided with two exchanges of females, which were discarded. From the fifth to the ninth day after irradiation they were paired with normal females, each of whose male Lyra offspring was utilized for the establishment of a line carrying one irradiated Lyra chromosome. Any newly arisen aberration could thus be localized in smears obtained from four to five larvae. Based on the experience of Lüning (1952) and Auerbach (1954) the time schedule was planned in order to utilize gametes irradiated during first meiotic prophase.

Thirty-five breaks were localized in the third chromosome (four in deficiencies, 16 in inversions, nine in translocations, six in complex aberrations). Six of these occurred in the immediate vicinity of the break points of the Payne inversion, but were not identical with them.

Applying a ruler to Bridges' salivary map (as reproduced in Sinnott, Dunn and Dobzhansky, 1950) distances of 5 mm, 10 mm and 25 mm, respectively, right and left of the four "Payne" break points were measured out. The number of breaks expected within these sections on the assumption of ran-

TABLE 1

Number of observed and expected breaks in different sections of Chrosomome III

Sections suspected of increased break incidence		(a) Chromosome lengths included in calculation: Total chromosome III (82.7 mm)	(b) Chromosome III excluding Hannah's break sensitive regions (61.6 mm)	(c) Chromosome III excluding only distal and central break sensitive regions (72.4 mm)
1. Hannah's (1951) break sensitive regions	o	8		
	e	9.1		
	p +	0.7 – 0.8		
2. ± 5 mm adjoining Payne breaks	o	5	4	5
	e	1.7	1.3	1.6
	p	0.03*	0.04*	0.02*
3. ± 10 mm adjoining Payne breaks	o	6	5	6
	e	3.4	2.6	3.2
	p	0.12*	0.12*	0.10*
4. ± 25 mm adjoining Payne breaks	o	8	7	8
	e	8.5	6.9	8.01
	p	0.9 – 0.8	0.95 – 0.90	

*Probabilities marked with an asterisk express the random chance by binomial *and* Poisson calculations, of obtaining the observed number of breaks or more. Probabilities without an asterisk were calculated by the χ^2 method (Yates' correction) and express the chance of obtaining the observed, or larger, deviations from random break distribution.

dom breakage was calculated and compared with the number observed (see table 1).

Pending control experiments with a homozygous coisogenic stock, these preliminary results may be compared with those of other workers who irradiated homozygous flies. The intrinsic break sensitive regions detected by several investigators have been mapped by Hannah (1951). As seen from row 1 of table 1, no preferential break location in Hannah's "sensitive sections" could be established in our experiment. Row 2 of the table shows that we observed a break preference for the regions including 5 mm map distance on either side of the "Payne" break points. This effect remains also significant when Hannah's break sensitive regions are excluded from the calculation (eliminating the Payne break point located in sensitive region 95). For the larger sections, including 10 and 25 mm, respectively, on either side of the Payne breaks (rows 3 and 4 of table 1), the number of hits observed by us corresponds very closely to expectation. We incline to the view that a more precise timing of the irradiation to meiotic (and possibly also gonial) prophase may show up even more clearly the postulated effect of pairing difficulties on restitution.

The hypothesis, if supported by more extensive work, may furnish the basis for the planned production of desired aberrations. It may also shed a new light on the role of structural heterozygosity in rapid evolutionary change.

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EFFECT OF SELECTION ON CROSSING OVER IN THE MALES OF *DROSOPHILA ANANASSAE*

An abnormally high rate of spontaneous crossing over in the males of *Drosophila ananassae* (a local population of Calcutta, India) was obtained in a series of elementary studies (Mukherjee, 1957, and Ray-Chaudhuri and Mukherjee, unpublished). Moriwaki (1937) and Kikkawa (1937) showed a lower but considerable frequency of spontaneous crossing over in the males of Japanese populations of *Drosophila ananassae*. The striking fact of crossing over in the males of this species was accompanied by unusually large variations in recombination values among different individuals. The present author (see Ray-Chaudhuri *et al.*, 1959) while studying the frequency of crossing over between two markers in the IIIrd chromosome (plexus, mutation for wing venation, symbol *px*; and peacock, mutation for wing morphology, symbol *pc*) observed a recombination range of zero to 25 per cent in different individual males, with an average value of 11.4 per cent (map distance between *px* and *pc*, according to Mukherjee, 1957, is 22.55 per cent). Attempts were made to select for different frequencies of recombination in males.

Selection was made along two lines. In one, the homozygous double recessives (that is, one of the parental types) were selected from those vials of test cross cultures which had shown no crossing over in the male and in another, homozygous double recessives were selected from those which had shown approximately 25 per cent recombination. After six to eight generations of selection the range of variation was found to be between zero to five per cent in the low line, and eight to 14.4 per cent in the high line (table 1), the average being only 1.5 per cent in the case of the former, and 12 per cent in the case of the latter.

Friesen (1936) found a range of variation of zero to 48 per cent in the recombination values in the x-irradiated males of *D. melanogaster* mostly with

TABLE 1

Results of selection experiments along low and high crossing over strains

Experiments	Number of IIIrd chromosomes tested	Range of recombination frequency	Average
		%	%
1. Original population of *px-pc*	1,216 (12 vials)	0 – 25	11.39
2. Low crossing over strain after 7 selections	10,061 (49 vials)	0 – 8	2.6
3. Low crossing over strain after 8 selections	8,433 (27 vials)	0 – 5	1.5
4. High crossing over strain after 6 selections	3,395 (17 vials)	10 – 27	14.3
5. High crossing over strain after 7 selections	1,238 (10 vials)	8 – 14.4	12.0

respect to the genes scarlet (*st*) and curled (*cu*), the map distance between *st* and *cu* being only six per cent. The average value in his case was only 3.2 per cent.

Similar types of selection experiments, with respect to crossing over in females of *D. melanogaster*, were previously done by Gowen (1919) and Detlefsen and Roberts (1921). Detlefsen and Roberts were able to isolate two different low crossing over strains for the loci white (*w*) and miniature (*m*), giving recombinations of 0.6 per cent and 6.0 per cent, respectively, but were unsuccessful in selecting for increased recombination strains (the normal recombination value between *w* and *m*, according to Bridges and Brehme, 1944, is 34.6 per cent). Recently Parsons (1958) has obtained increased recombination by selection for high crossing over in females of *D. melanogaster*, with respect to two markers, black (*b*) and purple (*pr*) from five per cent ± to eight per cent ± in nine generations (map distance between *b* and *pr*, according to Bridges and Brehme, is six per cent).

While the present experiments were successful in yielding a strain with very low crossing over in the males they were less so in the high selection line. In spite of selection for recombination in the range of 25 to 27 per cent the mean values remained in the range of eight to 14.4 per cent. Whether the greater success of selection for low recombination values was due to the occurrence of inversions, and what other mechanism underlies the limited success for high values, remain open problems (see Levine and Levine, 1954, 1955).

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CELLULAR ANTIGENIC DIFFERENCES BETWEEN INDIVIDUALS OF THE SPECIES *PEROMYSCUS MANICULATUS*

Erythrocytic antigenic differences between species of the genus Peromyscus and between populations of the species *Peromyscus maniculatus* have been previously demonstrated. Moody (1941) noted antigenic differences between *P. maniculatus* and *P. leucopus* using immune rabbit sera. Cotterman (1944) found that normal human group-A sera and group-B sera absorbed with pooled red blood cells of individuals of various species of the genus Peromyscus possess agglutinins showing quantitatively different reactions against various Peromyscus species. Moody (1948) demonstrated differential cellular antigenic components between populations of *Peromyscus maniculatus*. These cellular antigens were demonstrated using agglutinins produced by immunization of a rabbit with Peromyscus erythocytes. Pooled cells of various individuals of the species *P. maniculatus* were used by Moody for absorptions, thereby rendering the fractionation and isolation of unitary reagents nearly impossible.

Recently, the use of erythrocytes from individual mice of the subspecies *Peromyscus maniculatus gracilis* for heteroimmunization of rabbits and the subsequent absorption analysis of the immune rabbit serum has revealed two

antigenic components which quantitatively differentiate individuals of the subspecies *P. m. gracilis.*

Absorption analysis was performed using a total volume of approximately .25 ml. of packed washed erythrocytes from an individual mouse placed in equal volumes in three small tubes. Approximately .10 ml. of immune serum was absorbed step-wise through the series of three tubes. The final supernatant serum was tested against absorbing cells for completeness of absorption. The reagents thus prepared were tested for agglutinins against cells of other mice used for fractionation of the immune serum. Patterns of reactions between cells of various individual mice and absorbed reagents have demonstrated, presumably for the first time, apparently unitary saline (complete) agglutinin reagents. The *P. m. gracilis* tested have been shown to possess either or both of the antigenic types.

The antigenic types have been observed in individuals of *P. m. gracilis* from Alger County, Upper Penninsula, Michigan, and *P. m. bairdi* from Washtenaw County, Michigan, but are not present in the tested individuals of the species *Peromyscus leucopus* from Washtenaw County, Michigan.

To date definitive data regarding the mode of inheritance of these serotypes has not been ascertained, but preliminary breeding results tend to indicate that the antigenic types represent a two-allele, one locus system, genetically similar to the familiar human M-N system.

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VISUAL AND PHYSIOLOGICAL SELECTION IN CEPAEA

In a recent paper, Lamotte (1959) has suggested that the observed correlation between the varietal compositions of *Cepaea nemoralis* populations and their habitats can be due *either* to physiological differences between the genotypes, *or* to selection by predators. When discussing the types of selective forces acting to produce a correlation between composition and habitat, he says (p. 74), "Two explanations suggest themselves. The first one is based upon differences between the genotypes in such characters as vitality, fecundity, resistance to temperature and humidity, or preferences for these conditions according to the habitat. It is improbable that two different genotypes will react in exactly the same way in differing conditions of the environment. The second explanation is selection by predators, the various shell patterns insuring more or less natural protection." Again, (p. 83), "...Cain and Sheppard have emphasized especially the role of the visual selection by predators. But, as we have seen, there is also a physiological selection, which appears to be an agent even more effective than the visual selection."

These remarks show a considerable misunderstanding of our interpretation of the variation in Cepaea; this was immediately pointed out by one of us (P.M.S.) in the discussion following Lamotte's paper, but the whole of his contribution to the discussion was deleted by the editor. The statement by Van Valen (1960) that, in the unpublished discussion of Lamotte's paper, Sheppard agreed that Cepaea provides a probable example of drift in natural populations, as though there were no more to variation in Cepaea but this, is a fantastic misrepresentation and can only be given an unqualified contradiction. We stated in our first paper on Cepaea (1950, p. 287) that the most likely explanation of the polymorphism (as against those that ascribed virtually all variation in it to random fluctuation and/or an exceedingly high mutation rate (for example, Lamotte, 1951) was "a balance of different genetic forms in each population, maintained by the physiological advantage of certain gene combinations, but with the actual ratios of genotypes altered in different directions in different situations by the action of natural selection"—that is, by visual selection by predators acting on an initial balance, different in different regions, set up by physiological selection. We reiterated later (1954, p. 114) that "this snail is subject to strong visual selection by birds, which results in a correlation between the varietal composition of each colony and the exact background on which it lives. This selection must be balanced by strong non-visual selection, for the polymorphism to be maintained in each colony." Our view, therefore, has always been that in English colonies in equilibrium with their environment, non-visual selection must be more powerful than visual, and nothing has been published by authors working in other countries that would suggest either visual or non-visual selection to be unimportant elsewhere. (The possibility that the polymorphism is wholly maintained by visual selection, the commoner types being more heavily predated, was considered by us (1954) as much less likely.)

Lamotte's statement quoted above that physiological selection is "even more effective" than visual is ambiguous. Visual selection may well contribute more to the variance between colonies in relation to habitat, while physiological selection maintains polymorphism in each colony; the first can be more "effective" in producing inter-habitat variation, yet the second be more powerful, in that, in spite of the first, it maintains the polymorphism. However, Lamotte may mean that physiological selection contributes more to the between-habitat variance than does visual selection. This may well be truer in France than in England. He suggests (1959, p. 79) that the darker shells (pinks and browns as against yellows, and heavily banded ones as against unbanded ones) are most abundant in shady humid woodlands because of microclimatic selection. But we find (Cain and Sheppard, 1954) in English woodland colonies, that in the densest woods (beech and oak), unbanded and single-banded shells are commoner, in the more open ones with much scattered herbage and brambles, banded ones are commoner, as would be expected on the hypothesis of visual selection but not at all on Lamotte's hypothesis. Moreover, on sand-dunes by the sea, where insolation is certainly high, and many unbanded yellows would be expected on Lamotte's hypothesis, many pinks and heavily banded shells are found (Cain and Sheppard, in preparation). However, we certainly do not deny that some forms of microclimatic selection may have some effect in England, and quite probably much more in France.

In agreement with our suggestion (1950) that the physiological selection will act differently in different regions, Lamotte states that the frequency of different varieties does differ from region to region in France, and from France to England. Lamotte's experiments and those of others (see Lamotte, 1959) suggest that insolation is a more important selective agent in France than in England, and we agree with this. Nevertheless, it does not follow, as Lamotte claims, that visual selection is wholly unimportant in France. The detection of visual selection and the estimation of its importance in contributing to the variance between colonies merely by comparisons of the frequencies of varieties within predated and unpredated shells of the same colony is very difficult. Selective predation may be highly intermittent, and of different types at different seasons; consequently one must know the net effect over at least one complete annual cycle of predation (and more probably over several) in order to estimate the strength of selection. It is not allowable merely to collect one sample of broken shells, which may all be the result of rat-predation, or of winter predation by thrushes, or of visual predation by thrushes at one particular time during a seasonal change of background, when a given variety may even be temporarily neutral with respect to others.

The strength of visual selection of young snails by birds that do not leave behind the broken shells has never been determined. In thrush-predated colonies, the determination of visual selection merely by the thrushes requires an estimate of the total numbers taken per generation in relation to the size of the colony, and the net difference in varietal composition between

the predated and the non-predated snails. The contribution of the visual selection to the variance between colonies depends not only on the strength of the visual selection but also on the balance between it and the no doubt numerous and strong forces of physiological selection, which may differ considerably in different areas. It is not allowable, therefore, to estimate the importance of visual selection solely by determining whether there is a correlation between the direction of visual selection observed from predated shells, and the frequency of the favored type in each colony. Lastly, some colonies, recently disturbed or founded, may not be in equilibrium with their habitats, and in them no such correlation could be expected. The bulk comparison of predated and unpredated shells, without reference to any other data, therefore, can be most uninformative; more adequate methods must be used.

Moreover, Lamotte's argument from a lack of considerable correlation between the proportions of a given variety in *C. nemoralis* and in *C. hortensis* in mixed populations, that visual selection cannot be important, is incorrect, as we have already pointed out (1954). This argument depends on the physiological selection being the same in both species, which is certainly untrue, and on there being no interaction between banding and color because of visual selection, a point not considered by Lamotte. We have already demonstrated the high probability of such an interaction (1954); Clarke (1960) has now shown that if this interaction is taken into account, there is a strong correlation between different varieties in *nemoralis* and *hortensis* according to habitat. Where dark brown or pink unbanded shells are advantageous to *nemoralis*, *hortensis* camouflages its normal yellow by full banding with fusion of bands.

It is indisputable that in regions heavily disturbed by man, many colonies will be on recently changed backgrounds, and out of equilibrium with them; it is equally indisputable that in small colonies, and in large ones that have been founded only recently, there will be some effects caused by genetic drift or the founder principle. But the magnitude of these effects can be estimated only from a knowledge of the strengths of the selective pressures involved, and a detailed study of the geography and ecology of the colonies concerned. A mere comparison of the variances between populations in the classes of large and small populations is insufficient, since a greater variance between small ones can also be accounted for either by differing selection by predators owing to the greater heterogeneity between the backgrounds of small colonies, or by differences in physiological selection in less dense and more dense populations, or indeed by certain forms of simple visual selection.

As the result of recent studies by several authors, the interaction of visual and non-visual selection, mutation, migration, and genetic drift in *Cepaea nemoralis* and *hortensis* is becoming clearer, and geographical variation over large and small areas is being revealed. The principal need now is for good estimates of the selective values involved, so that the relative importance of other factors may be more firmly established.

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